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College of Veterinary Medicine
Department of Physiology, Biochemistry and Pharmacology

Protective Effect of α Lipoic acid(ALA) on Cardiovascular System of Male Rabbits Exposed to Mono Sodium Glutamate (MSG)

A THESIS

Submitted to the council of the College of Veterinary Medicine at the University of Kerbala as a Partial fulfillment of the Requirement for the Degree of Master in the Sciences of Veterinary Medicine /Physiology

BY

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2020 A.D **1441 A.H**

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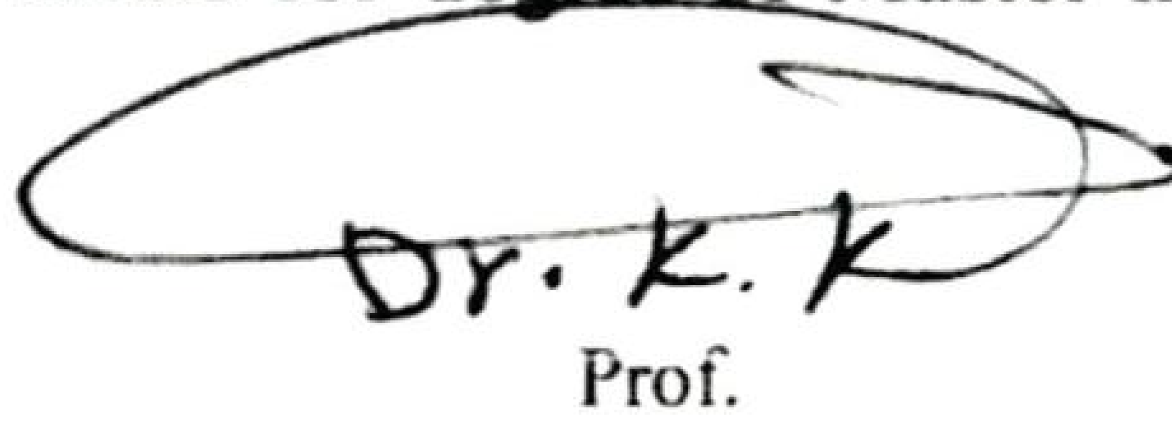

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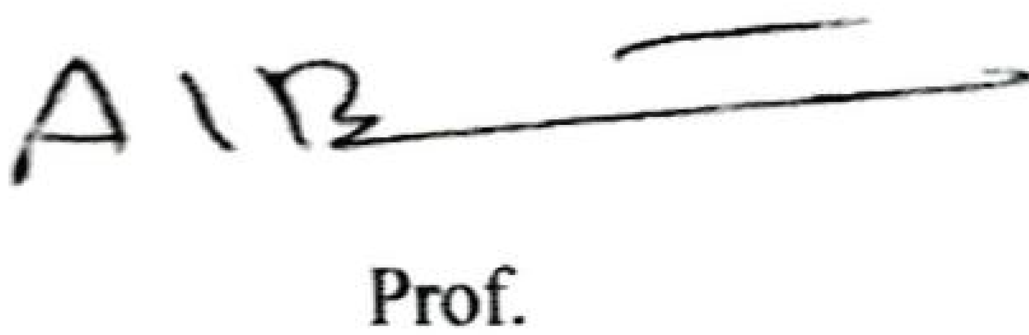

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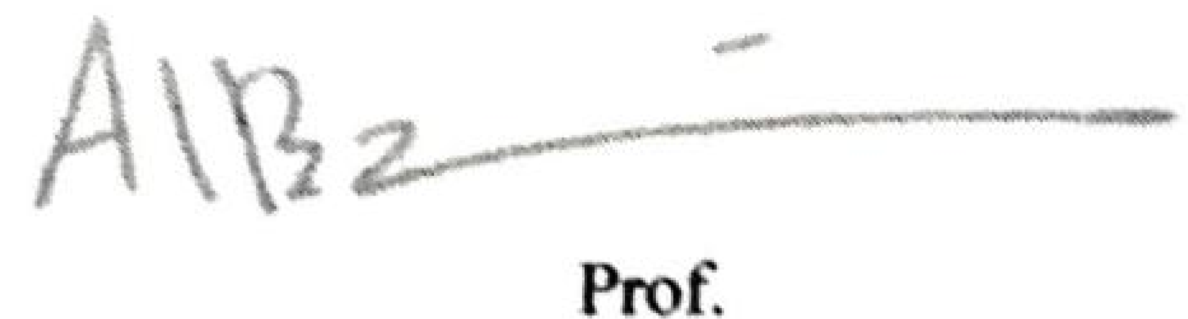

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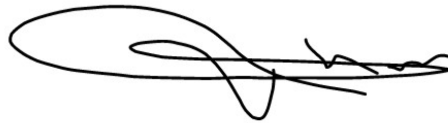

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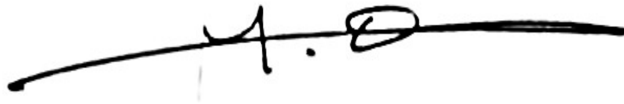

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Dedication

I dedicate this project to my two greatest blessing in my life. Allah and my mother

You are my life blessing .Thank you for being with me. My great mother.

To the symbol of sacrifice and my destiny in my life. my dear father.

To the love flows in my veins, and my heart . thanks for supporting me .My brothers (Saad ,Mustafa)

who have been constant source of help and encouragement . Our presence in my life is a Grace. My sisters (Suad, Maesson ,Sawsan ,Suha, Wasan , Sabreen)

To those who paved for me the way of science and knowledge.My teachers

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Eman

SUMMARY

Summary

The study was designed to investigate the protective role of Alpha Lipoic Acid (ALA)against Monosodium glutamate (MSG) induced heart and aorta damage in male rabbits . Some cardiac biomarkers were estimated in the serum, e.g. (Endothelin 1,troponin I and NO) and some enzyme activity (ATPase and NO synthase) ,Minerals (K, Na ,Ca) ,some oxidant (ONOO and MDA) and antioxidant (GSH) ,lipid profile (LDL-C,vLDL-C ,HDL-C ,TC, TAG)concentration , Electrocardiograph intervals (P, QRS and T) were monitored and histopathological examination of heart and aorta tissues were performed.

Thirty two adult male rabbits were randomly divided into four equal groups(8/group),The first group was intubated tap water and served as control group (GI).Rabbits in the second group were intubated orally with 8mg/kg B.W daily of MSG (GII),while animals of the third group were intubated with 60 mg/kg B.W daily of AIA (GIII). Animals in the last group (GIV) received 8mg/kg B.W of MSG and 60 mg/kg B.W daily for 10 weeks.

blood samples were collected by cardiac punctures technique at 10 weeks of experimental study to evaluate the possibility of recovery of heart and aorta tissue damage induced by MSG.

The result of our study indicates a significant ($p<0.05$)increase in the serum levels of ONOO, MDA and bad lipid profile (VLDL-C,LDL-C,TC,TG) with a significant increase in the serum level of Cardiac troponin I and Endothelin-1 and a significant increase in ATP synthase activity in animal group received MSG and a significant increase in [Na] hypernatremia with significant decrease of [K] hypokalemia , in addition the results revealed a significant ($p<0.05$) increase in the body weight and heart weight , while a significant decrease in the serum level

concentration of GSH, HDL-C and NO. The results of electrocardiogram records the current study showed the prolongation in the waves (P,QRS,T) and (ST,QT) intervals in the GII group compared to control and GIII,GIV groups .

Oral intubation of 60mg/kg B.W daily of ALA in GIII and together with MSG group GIV .The results showed an improvement recovery in heart and aorta finding through a significant($p<0.05$) decrease in the serum level of the ONOO, MDA ,bad lipid profile (LDL-C,VLDL-C,TC,TG). It also showed a significant increase($P<0.05$) in the serum level concentration in the GSH and NO compared to GI,GII groups .

Our result showed a significant ($p<0.05$)decrease in the serum Endothelin-1 and Troponin-I and ATP synthase ,serum activity and increase in the serum level of [K] and a significant decrease in the [Na] in GIII and GIV compared with another groups .The recovery role of the ALA in the electrocardiograph in the GIII and GIV showed a significant($p<0.05$)decrease in the (P, QRS,T)waves and (ST,QT) intervals compared to GII group.

The histopathological examination of heart and aorta in GII received MSG ,showed the heart appeared to have significant damage necrosis such as inflammation and fibrosis and infiltration of inflammatory cell, hemorrhage and congestion ,on the other hand thickening in aorta compared with other group and study shown protective role of ALA in Heart and aorta in GIII.

In conclusion , our study was conducted the injuries effect of MSG ,it should be avoided especially in heart and aorta disorder from foods containing excess MSG which can be fortified with orally intubation of 60mg/kg B.W of ALA to recover its adverse effects .

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LIST OF ABBREVIATION

Abbreviations	Meaning
μM/L	Micromolar per liter
ALA	Alpha lipoic acid
ATPs	Adenosine Triphosphate synthase
BMI	Body Mass Index
bt/m	Beats/minute
Ca	Calcium
cTnI	Cardiac troponin I
CVD	Cardiovascular disease
EDRFs	Endothelium dependent relaxation factors
ELISA	The Enzyme Linked Immuno Sorbent Assay
ET-1	Endothelin-1
FAD	Food and Drug Adminstration
GluRs	Glutamate receptors
Glut	Glutamate
GSH	Glutathione reductase
H&E	Hematoxylin and Eosin
HDL	High density lipoprotein
K	Potassium
LDL	Low Density lipoproteins
MDA	Malondialdehyde
mEq/l	Milliequivalent per liter

Mg/dl	Milligram per deciliter
mM/l	Millimoles per liter
MSG	Monosodium glutamate
MTKR	Massons trichrome
mv	Millivolet
Na	Sodium
Ng/l	Nanogram per liter
NO	Nitric Oxide
NOs	Nitric Oxide synthase
EDHF	Endothelin derived hyperpolarizing factor
GMP	Good Manufacture Practice
MMP	Matrix metallopeptidase
PARP	Poly-ADP ribose polymerase
MPK-1	Mitogen protein kinase-1
ERK	Extracellular signal regulated –kinase
EAATs	Excitatory amino acid transporters
MAPK	Mitogen activate protein kinase
s-GC	Soluble guan
ONOO	Peroxynitrate
ROS	Reactive Oxygen Species
S	Second
TC	Total Cholesterol
TG	Total glyceride
vLDL	Very low density lipoprotein

CHAPTER ONE

INTRODUCTION

1.introduction

Food additives are widely used to preserve the quality of the food ,to achieve the uniformity needed for large –scale production ,to enhance the flavor ,or to improve the texture of a food product **(Mahindru ,2004)**

Monosodium glutamate (MSG), one of the most common food additive used all over the world . The sodium salt of naturally occurring (non-essential) L form glutamic acid is a well-known food flavor enhancer. Its palatable and favorite flavor is a must in almost all Chinese and South-Asian dishes. L-glutamate is the molecule responsible for the umami taste (the 5th basic taste in addition to saltiness, sweetness, bitterness and sourness **(Amira *et al*.,2016)**. It is added to the food either as a purified monosodium salt or as a component of a mixture of amino acids and small peptides resulting from the acid or enzymatic hydrolysis of proteins **(Uneyama *et al*., 2009)**. Study recorded , the consumption MSG has increased all over the world **(**It is present in a wide variety of processed foods including flavored chips and snacks, soups or sauces (canned, packed) ,prepared meals ,frozen foods ,marinated meats ,fresh sausages ,bottled soy or oriented sauces ,and stuffed or seasoned chicken ,manufactured meats ,some hams ,luncheon chicken and turkey ,flavored tuna ,vegetarian burgers and sausages **Beyreuther *et al*.,2007)**.

Although there is no problem if MSG is present in small amounts in any one foods ,the problem moves to a much graver scale if small amounts in different common foods that are consumed daily. Moreover ,MSG might fall under different titles ,making it very difficult to determine what foods contain this additives**(Blaylock,2007)**.

Ingestion of high concentration of MSG causes the appearances of neurological disease ,mainly ,Parkinson and Alzheimer **(Arruda and ,Filho2003)**.

Previous animal studies have inference that MSG is toxic to different organs such as the brain (**Abass and Abd El-Haleem , 2011**), liver (**Ortiz *et al*., 2006**), kidneys (**Abass and Abd El-Haleem, 2011**), thymus (**Pavlovic *et al*., 2009)** reproductive system **(Bojanic *et al*., 2009 and Iamsaard *et al*., 2014)** .

Studies reported a correlation between MSG and overweight and hypersensitivity in the form of unpleasant symptoms such as numbness, tingling, headache, muscle tightness, general weakness, and flushing **(He *et al*.,)**
.

Furthermore , long-terms intake of MSG was shown to induce hyperphagia,obesity ,asthma, memory impairment ,and damage to hypothalamic neurons **(Von Diemen *et al* .,2006)**.Thus ,the addition of MSG to foods can ultimately be considered a health hazard .

Experimental study showed that prolonged consumption of MSG produced amyriad of toxic effects ,referred to as Chinese restaurant syndrome**(Paviovic and Sarac ,2010)**.This syndrome was characterized by sweating ,nausea ,headache, chest tightness ,and /or a burning sensation in the back of the neck**(Williams and Woessner,2009)**

Another study showed that MSG administration ,lipid peroxidation and a depletion of anti –oxidants in the thymus and spleen , a precursor to the pathogensis of many disease (**Zeinab *et al* .,2014**).They suggested that MSG induced oxidative stress in the thymus and spleen ,increasing their sensitivity to lipid peroxidation Oxidative stress and free radical is considered as the mechanism of MSG Agitate toxicities (**Singh and Ahluwalia, 2012**). It occurs attribute to imbalance between reactive oxygen species (ROS) produce and endogenous antioxidant defends mechanisms (**Griendling and FitzGerald, 2003**). And another study reported the toxic effect of MSG on the heart (**Kingsley *et al*., 2013**).

Metabolic therapy involves the administration of a substance normally occurring in the body in order to favorably influence metabolic reactions occurring within the cell , and it is an under –utilized method for the treatment of different heart diseases .This form of therapy differs fundamentally from standard cardiovascular pharmacological therapy by improving cellular energy production ,suppressing free radical generation **(Hadj *et al* .,2007).**

Alpha lipoic acid (ALA) has generated considerable clinical interest as a thiol –replenishing and redox-modulating agent**(Arivaszhagan *et al*.,2002).**

It is a natural antioxidant created in the mitochondria of the liver, kidney and other tissues which plays a crucial role in metabolism **(BETA *et al* ., 2015)**.It has been identified as a key antioxidant found naturally in our diets ,but it appears to have an enhanced function when given as a supplement. ALA can scavenge a number of free radicals in hydrophilic and lipophilic environments and found to be capable of regenerating many endogenous antioxidant in the body **(Biewenga *et al*.,1997)**.It has capable to prevent and treat of oxidative stress condition these include ischemia reperfusion injury**(Suzuki *et al*.,1992)** ,diabetes**(Henriksen *et al* .,1997)**,HIV infection **(Fuchus *et al*.,1993)**, and neurodegenerative disease **(packer *et al*.,1997)**.

LA is a hydrophilic and hydrophobic characteristics , being extensively distributed in plants and animals tissues in cellular membranes and in the cytosol. It is presently considered as one of the most effective cellular oxidation organized Regarding the strong antioxidant characteristics of lipoic acid, the protective role of LA on elected cardiovascular diseases.. previous studies have included that ALA causes protective role effect on kidney**(Faizah *et al*., 2005)** ,Diabetes **(Luc *et al* .,2015)**,testicular toxicity**(Chidambaram *et al* .,2005)** , reproductive organ **(Tahani *et al* .,2017)** . So the present study was examined to estimate the possible protective role of accompanist administration of ALA against cardiotoxic effect

of chronic oral intubation of MSG has been known to be one of the general oxidative stress and tissue damage in the heart and aorta on the male rabbits by the following :-

1.Estimation of serum

a.cardiac and blood vessels damage biomarker e.g.

Cardiac troponin-I, Endothelin-1 and Nitric Oxide (NO).

b. ATPsynthase and NOsynthase in the serum

c. Oxidant(ONOO,MDA)and antioxidant GSH.

d. Lipid profile (VLDL-C ,LDL-C ,HDL-C ,TC ,TG).

e. Cations [K^+,Na^+ and Ca^{++}]

2. Electrocardiographs recording (P,QRS and T waves with ST, QT intervals)

3. Histopathological change in the heart and large blood vessels (aorta).

CHAPTER TWO
LITERATURE REVIEW

2.Literature review

2.1 Mono Sodium Glutamate(MSG)

Achemical substance is present in the form of white crystals with achemical formula C5H8NO4NA. It is molecular weight 169.11g/ml. The boiling degree 232C °, water solubility 74g/100ml , commercial symbol 621E, It is one of the essential amino acid found in nature , Mono Sodium Glutamate contain 78% glutamate and 22% sodium and water , easily soluble in water and insoluble in organic solvent such as ethanol and . Non-hydroscopic compound is very stable as it has no change in appearance and quality during storage for long peroids at room temperature. It also does not decompose or rot but when the acidic condition and at high temperatures it dries relatively and turn into 5- pyrlidone -2 carboxylate as show in figure (2.1) **(Kaushalya and Jagath,2017).**

Monosodium glutamate (MSG), the sodium salt of glutamic acid, is widely used worldwide as an additive in foods to enhance their flavor by increasing taste perception. In addition, it has been highlighted out as a tool for reducing sodium in food products **(Beyreuther *et al*., 2007**).

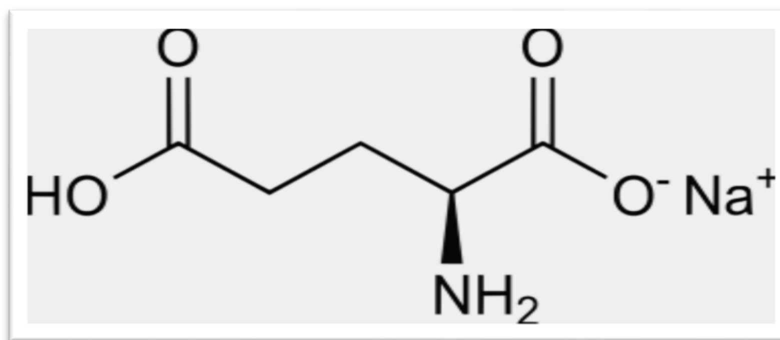
Glutamate is naturally present in the human body and has several important metabolic functions, which are mainly accomplished by regulating nitrogen metabolism and the energy supply and acting as a neurotransmitter in the central nervous system **(Kondoh *et al* .,2009).**

MSG was considered the safe by the Food and Drug Administration (FDA), but in fact it was exist that MSG reasons many health problems . Food and Drug Administration (FDA) declared glutamate as commonly recognized as Safe (GRAS) ingredient, along with other generally used food ingredients such as salt, vinegar, baking powder **(Geha *et al*., 2000).**

Mono Sodium Glutamate is used as a taste enhancer and is one of the most common food additives used to improve the taste of food . MSG the sodium salt of glutamic acid ,is widely used worldwide as an additive in food (snack ,sauces ,icecream ,gelatins ,pastry ,sweats, hardstack, Noodles, Juices, chocolate) to enhance their flavor by increasing taste perception **(Yamaguchi and Ninomiya, 2000).**

The umami taste substances are contained abundantly in various foods, including vegetables (e.g., tomato, potato, Chinese cabbage, mushroom, carrot, soybean and green tea), seafood (e.g., fish, kelp, seaweed, oyster, prawn, crab, sea urchin, clam and scallop), meat (e.g., beef, pork and chicken) and cheese, and contribute greatly to the characteristic tastes of these foods **(Kurihara ,2009)**.

If found to give flavor and tasty flavor to many food including soup and meat The ideal palatability concentration for MSG lies between 0.2 – 0.8% and its use tends to be self-limiting as over-use reductions palatability. The largest palatable dose for humans is about 60mg/kg body weight



Figure(2.1) chemical structure of MSG**(Kaushalya and Jagath,2017).**

The dicarboxylic, amino acid glutamate is a major oxidativefuel for the gut. In addition, glutamate is an important precursor for other biologically active molecules, including glutathione, proline and arginine, and also functions as a key neurotransmitter(**Reeds *et al* .,2000**) .

Several studies have shown that glutamate is extensively metabolized by the intestinal enterocytes. that only small fractions of luminally administered glutamate are absorbed into the mesenteric venous blood. dietary glutamate is extensively metabolized by the intestine and that oxidation to CO2 is a major metabolic fate. The oxidation to CO2 is a major metabolic fate of enteral glutamate even when the dietary intake fed is 3-4 fold higher than normal(**Janeczko *et al*.,2007**) . MSG causes many heart diseases such as Myocardial infaction,atherosclerosis **(Signora *et al* .,2012)**

Myocardial infarction

Acute coronary syndrome (ACS) represents arrange of ischemic heart disease from unstable angina to myocardial infarction , and may include large areas of cardiac necrosis **(Peela *et al* .,2010)**

Myocardial ischemia occurs when the oxygen supply to the heart is not sufficient to meet metabolic needs. This mismatch can result from a decrease in oxygen supply , a rise in demand , or both . the most common underlying cause of myocardial ischemia is obstruction of coronary arteries by atherosclerosis . Transient ischemic episodes are usually precipitated by an increase in (O2) demand as in exertion **(fauci , *et al*., 2010 and Surekha, *et al* .,2007)**

Atherosclerosis

Atherosclerosis has been derived from a Greek word , athero meaning gruel marche and introduced the term atherosclerosis describing the association of fatty

degeneration and vessels Stiffening . It the patchy intramural thickening of the subintima . The earliest lesion is the fatty streak . Fatty streak evolves to fibrous plaque and unstable plaque are responsible for clinical events**(Virmani, *et al*.,2006).**

Atherosclerosis is marked by atheromas , patchy initmal plaques , most common location is lumen of medium sized and large arteries . The plaque has cellular component –namely of inflammatory cells , smooth muscle cells , a fibrous component of connective tissue and a fat component of lipids . prominent risk factors of consideration are hypertension , diabetes , dyslipidemia , obesity , sedertory life style , family history , smoking intraplaque rupture , bleeding thrombosis and stenosis**(Aziz and Yadav,2016).**

2.1.1 Natural occurrence of Glutamate

Natural occurrence of Glutamate is one of the most common amino acids found in nature which is present in many proteins and peptides and most tissues. Glutamate is naturally synthesized in the body and binds with other amino acids to produce structural proteins **(Filer and Stegink,1994)**. When bound to protein molecules, glutamate is tasteless and it would not provide umami taste to food. Nevertheless, free glutamate is liberated in protein hydrolysis throughout fermentation, aging, ripening and heat cooking process **(Yoshida ,1998)**. In terms of foods like seafoods, meat, cheese and broths Glutamate is a crucial component of the taste **(Ninomiya ,1998)**. also reported measured free glutamic acid, which is present naturally in different foods, such as meat, poultry, seafood and vegetables **(Konosu and Hayashi ,1987).** Showed that by mixing umami taste substances,

amino acids and salt in appropriate ratios, the characteristic tastes of many natural foods could be reproduced.

2.1.2 Metabolism of MSG

From two main sources, humans are exposed to dietary glutamate. That is either from the digestion of ingested dietary protein or from the ingestion of foods that contain substantial amounts of free glutamate (naturally present, or added in the form of MSG/hydrolysed protein). Glutamate is absorbed in the gut by active transport system precisely for amino acids. This process is saturable and can be competitively inhibited and is dependent on sodium ion concentration**(Young and Ajami,2000)** .

Glutamic acid available in dietary protein is digested to allow amino acids and small peptides, both which are absorbed into mucosal cells where peptides are hydrolysed to free amino acids and some of the glutamate is metabolised. Additional amounts of glutamate appear in the portal blood, where it is metabolised by the liver. respectively confirmed that a majority of dietary glutamateis metabolised by the gastrointestinal tract. However, minute amount of dietary glutamate enters either the systemic or the portal blood supply **(Young and Ajami,2000)**, demonstrating it is almost exclusively utilised by the intestinal tissues. Furthermore, studies carried out by **(Stoll *et al* .,1998)** have revelaed that 95% of dietary glutamate presented to the mucosa was metabolised in first pass and that of this, 50% appeared as portal CO2, with lesser amounts as lactate and alanine which specifies that glutamate is the single largest contributor to intestinal energy generation. Further, it was evident that about 10% of dietary glutamate is incorporated into mucosal protein synthesis, with the balance being used for the production of proline, arginine and glutathione. Indeed, all three substances are derived exclusively from dietary glutamate, rather than the vast *in vivo* pool of

glutamate were first to show evidence of extensive metabolism of glutamine, glutamate, milk-based formula indicated that >95% of the dietary glutamine, glutamate, and aspartate are used in vivo by the gastrointestinal tract **(Stoll *et al* .,1999)**. glutamine as the main oxidative fuel in the gut. However, note that both glutamate and aspartate are of perhaps equal importance as intestinal oxidative fuels. Recent study in young pigs and humans confirms the extensive intestinal oxidation of dietary glutamate, glutamine, and aspartate **(Ricdijk and Goudoever,2007)**.

The intestinal metabolism of glutamate is presumed to occur largely in epithelial cells lining the mucosa, namely enterocytes. The first step in epithelial glutamate metabolism is transport from the intestinal lumen across the apical membrane. Glutamate transport by the enterocyte apical membrane occurs mainly by the high-affinity XAG system and to a lesser extent by the low affinity B'> system; the XAG system transports both glutamate and aspartate. The molecular identities of 4 proteins capable of XAG system activity have been described in various tissues, including glutamate-aspartate transporter I (GLAST-l), glutamate transporter 1 (GLT-l), excitatory amino acid carrier 1 (EAAC-l), and excitatory amino acid transporters 4 and 5 **(Beart and Oshea,2007)**. Study with pig and rodent tissues show that EAAC-I is the most abundant glutamate transporter in the intestine and is expressed on the apical, brush border membrane throughout the small intestine the expression of EAAC- 1 in isolated epithelial cells all along the villus and crypt, and this has been supported by immunohistochemical analysis showing expression in the brush border membrane **(Iwanaga and Goto,2005).**

The latter study reported that EAAC-1 expression was localized mainly to the small intestine and was not highly expressed in the stomach or large intestine. However, both the GLAST and GLT- I transporters were expressed in various cell compartments within the stomach and to a lesser extent in the small intestine, once

inside the intestinal enterocyte, glutamate catabolism occurs in the cytosol and mitochondria by transamination by aspartate aminotransferase, alanine aminotransferase, branched chain aminotransferase, and glutamate dehydrogenase (GDH) enzymes, all of which are present in the stomach, small intestine, and colon **(Sweatt *et al* .,2004)** . Interestingly, the activity of GDH is increased approximately 3-fold in the small intestine after weaning in piglets and rats **(Madej and Lundh, 2002)**. The resulting keto-acid product of branched-chain aminotransferase and GDH is a-ketoglutarate, which can then enter the tricarboxylic acid cycle and be metabolized, yielding carbon dioxide. studies with perfused rat intestine and those in vivo with piglets and humans indicate that most of the glutamine (55-70%), glutamate (52-64%), and aspartate (52%) are oxidized to carbon dioxide as in figure (2.2) **(Douglas and Barbara,2009).**

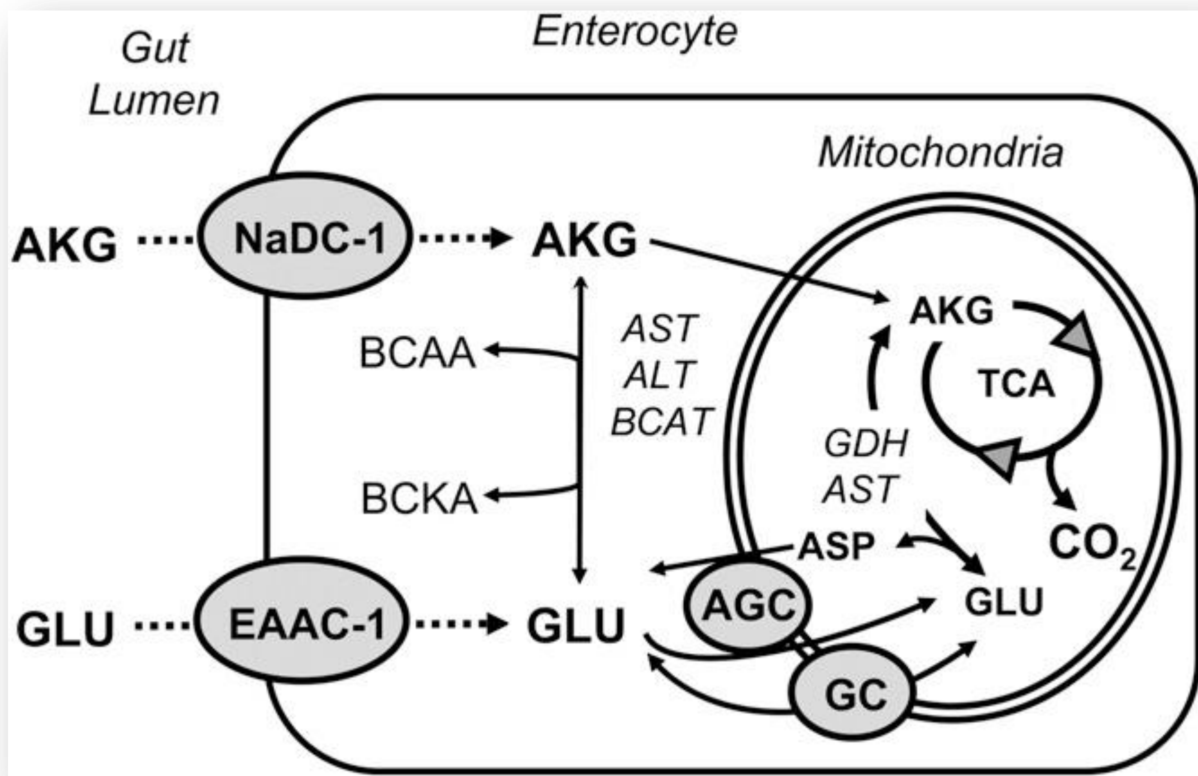


Figure (2.2) Metabolic fate of dietary glutamate (GLU) and a-ketoglutarate (AKG) in the intestinal enterocyte. Dietary GLU and AKG are transported from the gut lumen into the enterocyte by the excitatory amino acid carrier-I (EAAC-l) and Na-dicarboxy late cotransporter-1 (NaDC- I) transporters, respectively. Within the enterocyte, both GLU and AKG can undergo transamination and transport into the mitochondria for oxidative metabolism to CO,. BCAA, branched-chain amino acid; BCKA, branched-chain keto acid; AST, aspartate aminotransferase; ALT, alanine aminoti-ansferase; BCAT, branched-chain aminotransferase; TCA, tricarboxylic acid; GDH, glutamate dehydrogenase; AGC, aspartate-glutamate carrier; GC, glutamate carrier **(Douglas and Barbara,2009).**

2.2 Anatomy of rabbit heart

It is placed high in the chest ,between the lungs and near the sternum, The size of the rabbit heart is relatively small in relation to the size of its body, and in comparison with that of other animals .protected by the pericardium. This double-walled sac consists of a deep layer (visceral pericardium) and a superficial layer (parietal pericardium) between which lies the pericardial cavity filled with fluid. The sliding of the sheets relative to each other allows the movement of the heart. The pericardial fluid, in turn, protects the heart against shocks. The membrane of the pericardium is attached to the diaphragm, which helps keep the heart and major blood vessels in position in the thorax. The axis of the heart runs along the ventral chest, slightly deviating to the left side. The heart shape is conical, the wider part or base is directed towards the front of the thorax while the apex (bottom tip) is directed to the spine and slightly to the left as in figure (2.3) **(Esther ,2015)**

The base is directed forwards. As observed in other small animals, the rabbit heart has 4 chambers: 2 auricles and 2 ventricles separated by inter-auricular and inter-ventricular septa. Right and left ventricles that form the muscular caudal portion of the rabbit heart. The left ventricle is larger than the right one; they are separated from each other by the interventricular septum. Their walls are raised into muscular ridges. The right ventricle is much thicker than the right auricle and forms the right side of the conical apical portion, but without reaching the apex. It gives off the pulmonary artery in front. Cusp valves separate the ventricular chambers from the pulmonary artery and the aorta.

The ventricular chambers are separated from the auricles by flap valves, which are held in place by tendons. The heart valve between the right auricle and right ventricle (tricuspid valve) possesses only two cusps, an not three as usually observed in other animals.

Right and left auricles, which are located in the cranial part of the heart. They are small chambers that receive the venous blood from: The cranial and caudal vena cava (one of two large veins returning blood from outer parts of the body to the right chamber of the heart) and the coronary sinus (receiving blood from the heart itself), which are drained into the right auricle,

The left and right pulmonary veins that bring oxygenated blood from the lungs, open together into the cavity, on the dorsal side of the left auricle. Each auricle possesses, in addition, small muscular flaps sino-atrial node - or pacemaker from which originates the heartbeat - is located in the wall of the right auricle**(Bray *et al*., 1992).**

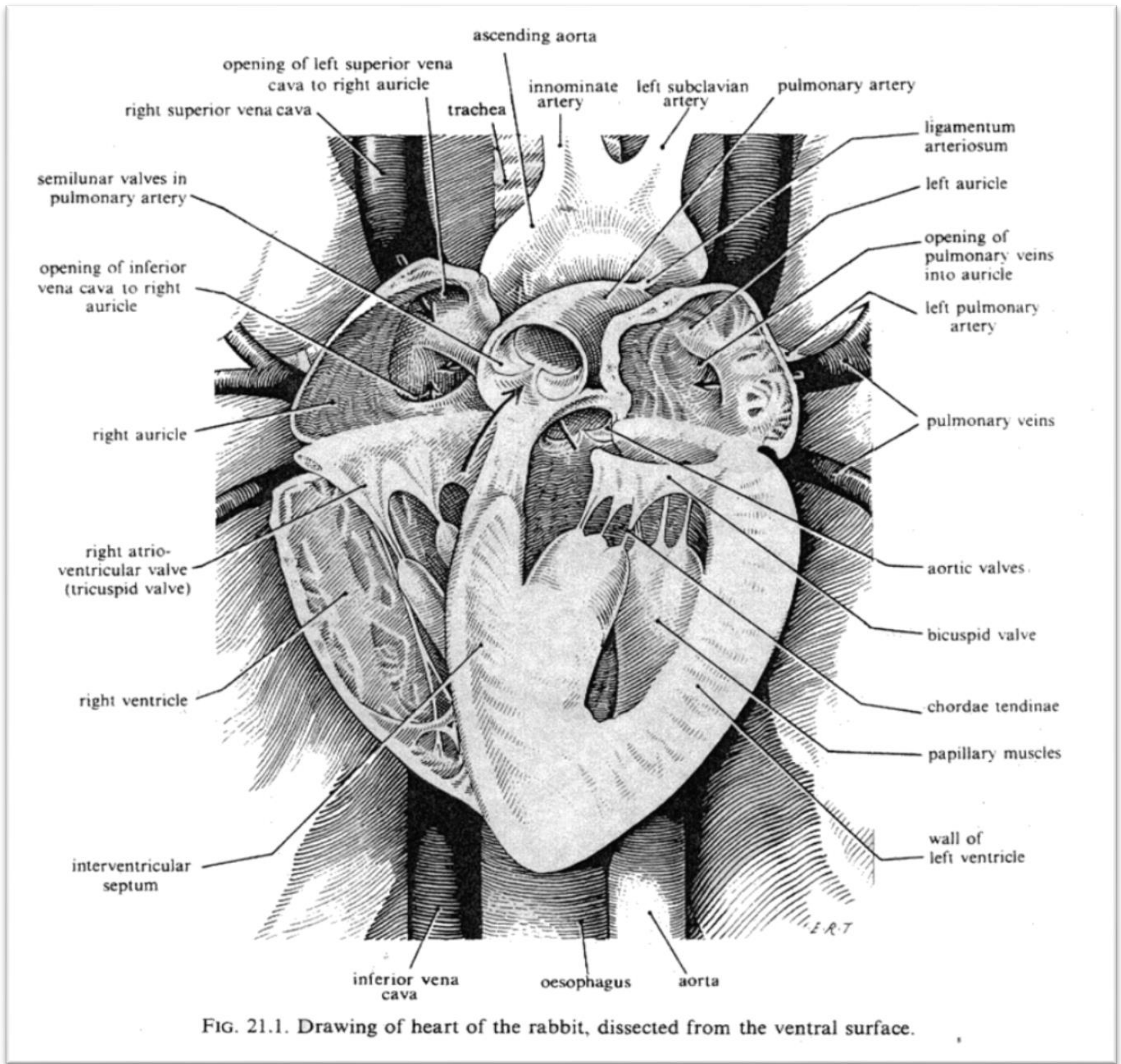


FIG. 21.1. Drawing of heart of the rabbit, dissected from the ventral surface.

Figure (2.3)Anatomy and physiology of rabbit heart **(Esther ,2015).**

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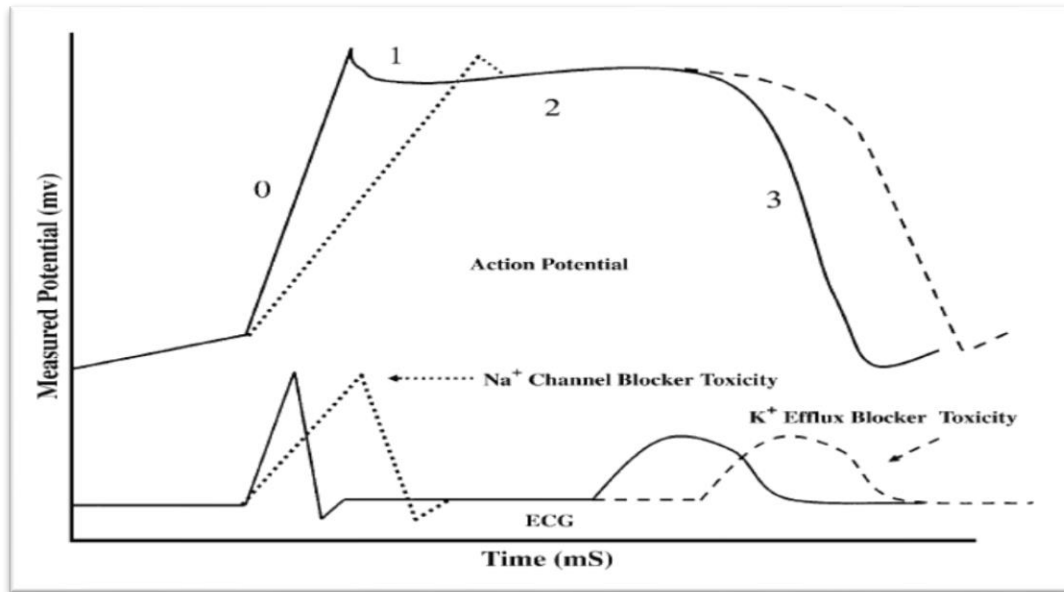
2.2.1 Physiology of cardiac muscle

Myocardial cells are branches and interdigitates with adjacent cells .An intercalated disc permits electrical conduction via gap junction. the basic unit of contraction is the sarcomere (2um long), which is aligned to those of adjacent myofibrils, giving a striated appearance due to the Z_lines .Actin filaments are attached at right angles to the Z_lines and interdigtate with heads of thicker myosin molecules which contain myofibrilar adenosine triphosphatease (ATPase). The cross –links between actin and myosin which breaks down adenosine triphosphate (ATP) to provide the energy for contraction .Two chains of actin molecules from ahelical structure, with asecond molecule, tropomyosin, in the grooves of the actin helix, and a further molecule complex, troponin, attached to every seventh actin molecule. During the plateau phase of the action potential, calcium ions enter the cell and are mobilized from the sarcoplasmic reticulum. They bind to troponin and there by precipitate contraction by shortening of the sarcomere through the interdigitation of the actin and myosin molecules. The force of cardiac muscle contraction , or inotropic state, is regulated by the influx of calcium ions through , slow calcium channel, the extent to which the sarcomere can shorten diameter stroke volume of the ventricle **(Boon *et al* .,2010).**

The myocardial cell membrane in its resting state is impermeable to Na+ .The Na+/K+ ATPase actively pumps three sodium ions out of cardiac cells while pumping in two potassium ions to maintain a negative electric potential of approximately 90 mV in the myocyte (phase 4). Depolarization of the cardiac cell membrane is caused by the rapid opening of Na+ channels and subsequent massive Na+ influx (phase 0). This Na+ influx causes the rapid upstroke of the cardiac action potential as it is conducted through the ventricles and is directly responsible for the QRS interval of the ECG. The peak of the action potential is marked by the

closure of Na+ channels and the activation of K+ efflux channels (phase 1). Calcium (Ca++) influx then occurs, allowing for a plateau in the action potential (phase 2) and continued myocardial contraction. The cardiac cycle ends with closure of the Ca++ channels and activation of K+ efflux channels, causing the potential to again approach _90 mV (phase 3). It is this potassium efflux from the myocardial cell that is directly responsible for the QT interval on the ECG as show in figure (2.4) **(Yap and Gamm,2003).**



Figure(2.4) Cardiac cycle action potential with corresponding electrocardiographic tracing. Dotted line indicates the changes associated with Na+ channel blocker toxicity. Dashed line indicates the changes associated with K+ efflux blocker toxicity**(Yap and Gamm,2003).**

2.2.1 Metabolism of cardiac muscle

Cardiac muscle depends almost exclusively on aerobic respiration to make ATP , it is very rich in myoglobin and glycogen (stored energy) ,and also has especially large mitochondria, which fill about 25% of the myocyte . Cardiac muscle is relatively adaptatable with respect to the organic fuels used . AT rest , the heart gets about 60% of its energy from fatty acids, 35% from glucose , and 5% from

other fuels such as ketones ,lactic acid , and amino acids. Cardiac muscle is more vulnerable to an oxygen deficiency than it is to the lack of any specific fuel . Because it makes little use of anaerobic fermentation or the oxygen debt mechanism , it is not prone to fatigue **(Saladin,2004)**

The basal O2 consumption by the myocardium is about 2 milliter /100 gram /minute (mL/ 100 g/min). This value is considerably higher than that of resting skeletal muscle . Oxygen (O2) consumption by the beating heart is about 9 ml/100/g/min at exercise . Increase occur during exercise and in a number of different states.

Oxygen demand is a major factor in local coronary blood flow regulation. Blood flows in the coronaries usually is regulated almost exactly in proporation to the need of the cardiac musculature for oxygen . The coronary blood flow does increase almost in direct proportion to any additional metabolic consumption of oxygen by the heart **(Guyton and Hall ,2006).**

2.3 The Electrocardiograph (ECG)

The collective spread of action potentials through the myocytes produces small voltages that can be measured on the surface of the body with an ECG. A standard ECG is obtained by placing an electrode on each limb . In a lead, one electrode is regarded as the positive side of a voltmeter and another is the negative side. A lead reports changes in voltage difference between the positive and negative electrodes. By varying which electrode is regarded as positive or which is negative, a standard leads provides a range of views of electrical events in the heart. illustrates the major waves on an ECG, together with standard intervals and segments and standard calibrations of time and voltage.

Electrical activity in nodal and conducting tissue is not seen on an ECG because the amount of tissue is too small to produce measurable voltage differences at the body surface ECG records . To obtain these recordings, electrodes were placed on the left forelimb, right forelimb, and left hind limb. Electrodes on these limbs are usually envisioned as forming a triangle around the heart .

The various ECG tracings in were obtained by interconnecting these electrodes in standardized combinations prescribed by Willem Einthoven, inventor of the ECG. the left forelimb compared with the right forelimb is called lead I. The same pattern of distinct P, R, and T waves is evident in the lead I (although the T wave happens to be negative In accordance with Einthoven's convention, the connections for the three standard limb leads in the form of a triangle (Einthoven's triangle). The triangle indicates that to make a lead I ECG, the voltage is recorded in the left forelimb (labeled the + electrode) compared with the right forelimb (called the − electrode). Similarly, the diagram indicates that lead II is the voltage measured in the left hind limb compared with the right forelimb, and lead III is defined as the voltage in the left hind limb compared with the left forelimb. It is important to remember that the + and − signs on Einthoven's triangle are simply notations about how to hook up the electrodes. That lead I is obtained by measuring the voltage in the left forelimb compared with the right forelimb (not vice versa).

The + and − signs on the triangle do not necessarily correspond to the orientation of the dipoles created in the heart , the major ECG events (P, R, and T waves) are normally evident whether one is looking at tracings from leads I, II, or III. These standard limb leads simply provide different angles for viewing the electrical dipoles created by the heart muscle as it depolarizes and repolarizes. Three additional electrical views are provided by the augmented unipolar limb leads (aVR, aVL, and aVF). Lead aVR measures the voltage from the right

forelimb electrode compared with the average voltage from the other two limb electrodes. Similarly, aVL and aVF measure the voltages from the left forelimb and left hind limb electrodes compared with the average voltage from the other two electrodes. The electrocardiogram (ECG) is made up of a wave that represents the electrical voltage produced by the contraction of atrial muscles by generating a depolarization of atrial muscles cell (P wave) and the (QRS) complex that causes the generating of ventricular depolarization and (Twave) that cause the generated by the repolarization of the ventricular muscle **(Jonathan *et al* .,2009)** .

Leads I, II, and III are used routinely in veterinary electrocardiography. Recordings from the augmented unipolar limb leads (aVL, aVR, and aVF) are often included as well. Special additional leads are sometimes recorded by placing ECG electrodes at standardized sites on the thorax. These *precordial (chest) leads* are used more often in human medicine than in veterinary medicine. They are helpful in evaluation of very specific cardiac electrical dysfunctions. The standardized vertical calibration on an ECG is that two major divisions equal 1 millivolt (mV). Two standard chart speeds are used: 25 millimeters per second (mm/sec), whereby five major divisions on the horizontal axis (time) equal 1 second; or 50 mm/sec, whereby 10 major divisions on the horizontal axis equal 1 second. Using the faster chart speed (50 mm/sec) helps to spread out the ECG events in an animal with a rapid heart rate . Chart speed is a convention derived from older, analog, paper-readout (strip chart) ECG machines. Although ECG is now more commonly captured and stored digitally, the chart speed convention is still used to set the resolution of the digital display. Furthermore, many of these digital units can produce a permanent paper printout of their data that looks just like the older strip chart as show in figure(2.5) (**Bradely and Kleing ,2003**).

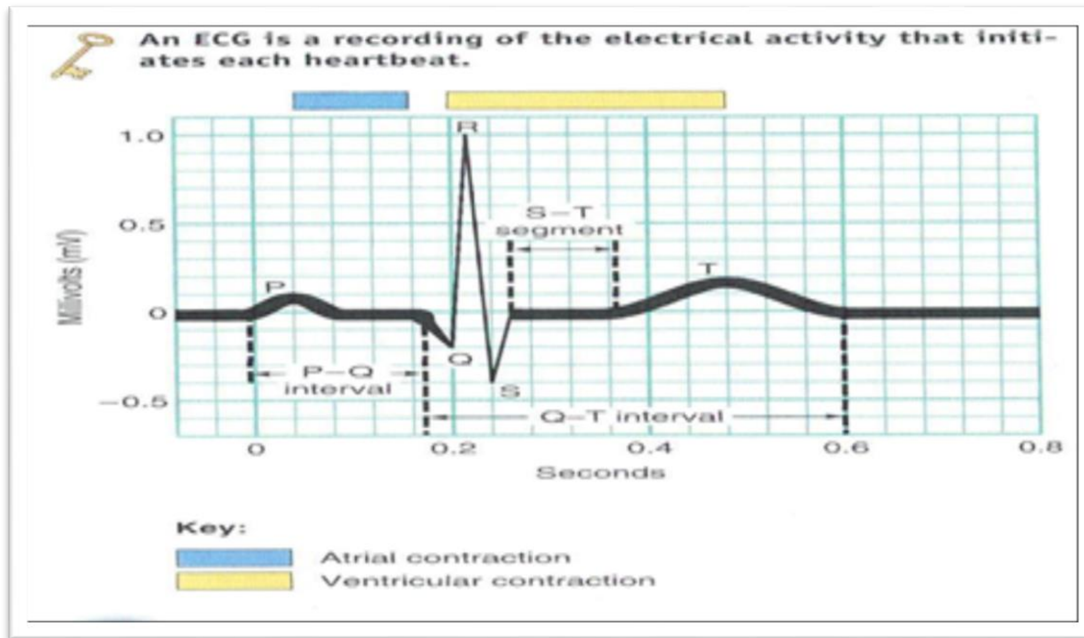


Figure (2.5) electrocardiograph is a recording of the electrical activity(**Bradely and Kleing ,2003**)

The ionic content of extracellular fluid, its historical derivation from its primitive forerunner sea water, and the importance of changes in its composition to the economy of the individual have been portrayed lucidly . The force and general behaviour of cardiac contraction can be altered in striking fashion under experimental conditions by varying the physical and chemical composition of the fluid bathing the heart muscle. Slight increases in alkalinity or acidity bring about a lengthening or shortening of systole respectively and influence the transmission of the impulse in the conduction system.

2.4 Alpha lipoic acid (ALA)

ALA is a specific antioxidant; it can easily quench radicals, has an amphiphilic character, and does not exhibit any serious side effects **(Gora *et al.*, 2011)**. ALA a compound that contains sulfur in the form of two thiol groups .ALA is also called thioctic acid and chemically symbol ($C_8H_{14}O_2S_2$) with an oxidized (disulfide,

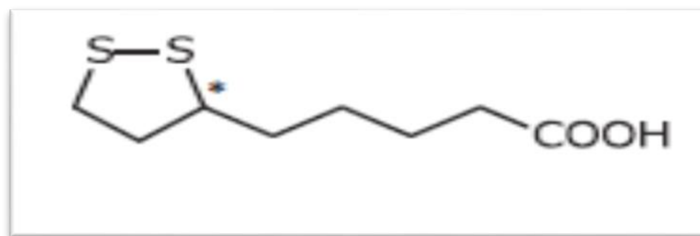
LA) and with a reduced (di-thiol: dihydro-lipoic acid, DHLA) form of LA as show in figure (2.6) **(Luc Rochette *et al* .,2015) .**

ALA acts as a cofactor for several mitochondrial enzymes by ability to directly scavenge ROS, its metal chelating activity, and its potential to react with, and regenerate, other antioxidants such as glutathione and vitamins E and C **(Singh and Jialal ,2008)**.

ALA also demonstrates anti-inflammatory properties.An additional advantage of ALA is its solubility both in water and in fat, which allows it to travel to all parts of the body. Because of its special properties, it is able to enter certain parts of the cell that most other antioxidants are not able to reach. This compound acts by many mechanisms and can therefore be a very effective antioxidant. Hence, ALA is used in various diseases concerning age-dependent oxidative stress. It can be particularly effective in cardiovascular diseases, including ischemic heart disease, hypertension, heart failure, and atherosclerosis, where it may slow aging and prolong lifespan. Many studies have confirmed that ALA can improve vascular function and decrease the atherosclerotic plaque burden (**Wollin and Jones, 2003**).

ALA inhibits the vascular overproduction of endothelin I, the main vasoconstrictor**(Takaoka *et al* .,2001)**. Furthermore,ALA significantly increases the synthesis of NO, the main vasodilator; it may also improve the redox state of the plasma and improve endothelium-dependent NO-mediated vasodilation. In addition, LA ameliorates the loss of eNOS phosphorylation, which contributes to improved endothelial Function. ALA act as a defensive agent versus risk factors of cardiovascular disease and may influence the CVD risk *via* the beneficial actions on LDL oxidation, blood lipid profiles, plaque formation and hypertension (**Wollin and Jones,2003**). ALA has the ability to scavenge ROS, metal chelating, and regenerate, glutathione, vitamins E and C **(Singh and Jialal,2008)**. ALA also has anti-inflammatory properties. ALA can mend vascular function and decrease

the atherosclerotic plaque burden. . DHLA, the reduced form of ALA, is capable of exerting an antioxidant effect directly by donating electrons to a pro-oxidant or an oxidized molecule. It can regenerate ascorbic acid from dehydroascorbic acid, and it can indirectly regenerate vitamin E back from its oxidized state . As well, ALA metabolites have been shown to have antioxidant effects **(Kwiecien *et al*.,2013)**. ALA, the oxidized form of DHLA, can exert an antioxidant effect. But this does not mean there is any donation of electrons by ALA to a pro-oxidant or oxidized molecule, since there are none to give. However, it has been documented that ALA can inactivate free radicals, which is a significant antioxidant effect**(Packer *et al*.,2001)**. Also, the ability of ALA to chelate metals can produce an antioxidant effect . DHLA can exert a pro-oxidant effect of donating its electrons for the reduction of iron, which can then break down peroxide to the prooxidant hydroxyl radical *via* the Fenton reaction . So, ALA and its reduced form DHLA, can promote antioxidant properties. ALA has been effectively chelate toxic metals directly, and it also indirectly strongly supports the chelation of metals by its ability to increase glutathione levels inside the cells**(Ghibu *et al*., 2009)**



Figure(2.6) Chemical structures of (ALA) **(Luc Rochette *et al* .,2015) .**

2.5 Nitric oxide (NO)

Nitric oxide is a gaseous biological mediator that accounts for the vasodilator activity of endothelium-derived relaxing factor (EDRF) , a non-prostaglandin vasorelaxant substance first described in the endothelial cells , it is generated from the guanidino-nitrogen of L-arginine yielding citrulline **(David *et al* .,2003)**, and plays a prominent role in controlling a variety of functions in the cardiovascular,immune, reproductive, and nervous systems **(Marletta,1994)**. Nitric oxide (NO) has an important role in myometrial function showing that NO generated by nitric oxide synthase (NOS) from arginine has a relaxant effect on the myometrium and that this relaxation is specifically blocked by inhibitors of NOS(**Jassim *et al*.,2000**).

Nitric oxide production is catalyzed by three major isoforms of NO synthase (NOS), neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) enzymes. The nNOS and eNOS are considered to be constitutively expressed and activated by calcium entry into cells, whereas iNOS is calcium-independent, and its synthesis is induced in inflammatory and other cell types by stimuli such as endotoxin and proinflammatory cytokines. Although cDNAs for the respective proteins are found almost in all mammalian cells, under physiological conditions,eNOS is the major NOS isoform expressed in the endothelial cells . In contrast, inflammation and cell damage are often associated with the expression of iNOS (**Radomski and Salas,1995**).

In the heart, both, eNOS and iNOS, have been involved in signalling pathways that modulate the contractile properties of cardiac myocytes. The eNOS isoform is expressed within the heart in the endothelium both of the endocardium and of the coronary vasculature, in cardiac myocytes, and in specialized cardiac conduction tissue and its activity seems to be regulated by the contractile state of the heart . In

contrast, iNOS expression is induced by cytokines in cardiac myocytes, endocardial endothelium, infiltrating inflammatory cells, vascular smooth muscle, fibroblast, and microvascular endothelium **(Balligand *et al*.,1994)**.

Nitric oxide is generated and released from the endothelial cells both under basal and agonist stimulated conditions. Shear stress and pulsatile flow are major stimuli that cause release of NO under basal conditions . In the cardiovascular system, NO not only causes vessel relaxation, but also inhibits platelet adhesion and aggregation, smooth muscle cell proliferation, monocyte adhesion,expression of different adhesion molecules and ET-1 production . The effects of NO on myocardial functions are still a matter of extensive investigation. There is now evidence showing that the basal endogenous NO production supports myocardial contractility and heart rate, whereas the expression of iNOS has been reported to have cardiodepressive actions because of the negative inotropic effects of NO at high concentrations**(Kojda and Kotteenberge ,1999)**.

2.6 Cardiac troponin I

Troponin I is a subunit of the troponin complex (Tn), which is a heteromeric protein that is bound to the thin filament. The troponin complex plays an important role in the regulation of skeletal and cardiac muscle contraction. The complex consists of three subunits: troponin T (TnT), troponin I (TnI) and troponin C (TnC). These subunits are held together by non-covalent interactions. TnT is the tropomyosin-binding subunit that regulates interaction between the troponin complex and the thin filament. The TnI subunit is responsible for inhibiting actomyosin formation at low intracellular Ca2+ concentrations. The TnC subunit binds Ca2+ ions during the excitation of the muscle and changes the conformation

of the troponin complex, thus enabling the formation of actomyosin complex and thesubsequent muscle contraction **(Gomes , 2002)** .

Troponins are regulatory proteins that are part of the contractile apparatus of skeletal and cardiac muscle tissue(**Scott *et al* .,2008**). They are not present in smooth muscle tissue. With the proteins actin and tropomyosin, they are part of the thin filaments within the myofibrils and are essential for the calcium-mediated regulation of muscle contraction. The troponin complex consists of 3 interacting and functionally distinct proteins (troponin I, T, and C) Tissue-specific isoforms exist for each type of troponin. Within the thin filament, tropomyosin dimers form a continuous chain along the groove of the actin helix. The troponin complex lies at regular intervals along the filament. Tropomyosin acts to block the myosin binding sites on actin. Each troponin protein has specific functions that regulate muscle contraction.

Troponin C (TnC) is present in 2 isoforms. One isoform is present in fast-twitch muscle fibers and the other is present in both cardiac and slow-twitch muscle fibers. Homology between the cardiac isoform and 1 of the skeletal muscle isoforms reduces the cardiac specificity of TnC and therefore limits its diagnostic usefulness in heart disease. Troponin C binds calcium to initiate muscle contraction.

Multiple isoforms of troponin T (TnT) exist in skeletal muscle. Cardiac troponin T (cTnT) has a molecular weight of 37,000 Da.10 In human cardiac tissue 4 isoforms exist, but only 1 is characteristic of the adult heart. The other 3 cardiac isoforms are expressed in fetal tissue. The fetal isoforms may be re-expressed during heart failure or in damaged skeletal muscle. Troponin T attaches the troponin complex to tropomyosin and actin **(Filatov et al.,1999)**.

Three isoforms exist for troponin I (TnI), two are present in skeletal muscle and the other is present only in cardiac muscle,the cardiac isoform (cTnI) with a

molecular weight of 24,000 Da, is larger than the other isoforms as it contains an additional 32 amino acid Nterminal peptide. The rest of the protein has greater than 40% dissimilarity in its amino-acid sequence compared with skeletal muscle TnI(**Babuin and Jaffe,2005)** .

Unlike cTnT, cTnI is not expressed in fetal skeletal muscle during development, nor after damage and regeneration in adult skeletal muscle. Troponin I inhibits actomyosin ATPase and prevents the structural interaction of myosin with actin-binding sites. The binding of calcium to troponin C displaces troponin I and causes a conformational change in tropomyosin so that it no longer interfere0 with myosin/actin binding and muscle contraction can occur as show in figure (2.7).

Troponin release kinetics are consistent with 2 separate intracellular populations. After acute cardiac injury, the cytosolic pool is released resulting in an early rise in blood levels. This is followed by the slower release of structurally bound troponin that results in a sustained elevation **(Jaffe *et al*.,1996)**.The half-life of troponin and its complex in the circulation is about 2 hours. In humans with acute myocardial infarction (AMI), cTn levels begin to rise 4–12 hours after the infarction and reach peak values at 12–48 hours. The levels remain elevated for 7–10 days (cTnI) and 10–14 days (cTnT)**.(Goldmann *et al*.,2001)**

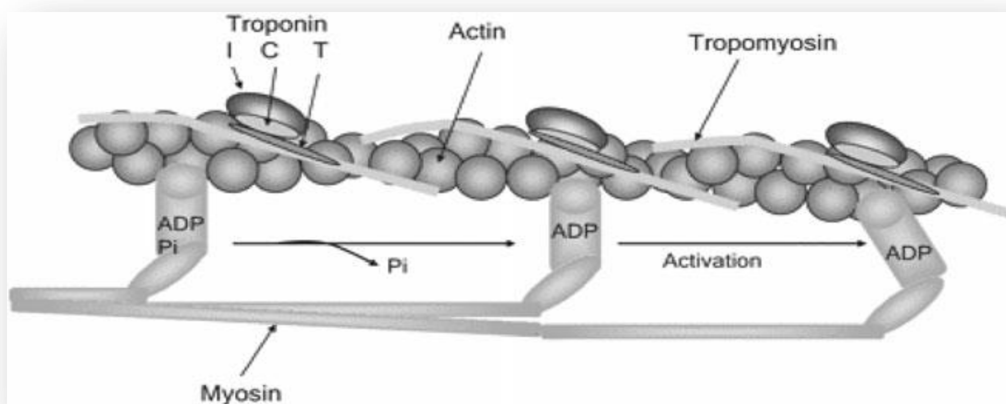


Figure (2.7) Detail of the interaction between thin (actin) and thick (myosin) filaments. Adjacent to each myosin-binding site on the actin chains there is the troponin complex composed of T (tropomyosin-binding), I (inhibitory), and C (Ca binding) subunits**(Juliet,*et al*.,2008).**

2.7 Endothelin-1

ET-1 is the main cardiac endothelin produced by cardiomyocytes, endothelial cells, and cardiac fibroblasts. It is interesting to note that ETA receptors represent most of the Endothelin receptors present on cardiomyocytes. The endothelin system in the heart seems to affect inotropy and chronotropy, but also mediate cardiac hypertrophy and remodeling in congestive heart failure through its mitogenic properties **(Miyauchi and Masaki ,1999)**.

Endothelin-1 (ET1) is a 21-residue vasoconstrictive peptide , Endothelin peptides are resulted in multiple tissues, and they represent as modulators of vascular tone, cell proliferation and hormone production . Endothelin-1 (ET-1) is concerning to endothelial dysfunction, and other cells are also able of output the ET-1, such as vascular smooth muscle cells , mesangial cells of the kidney **(Caires *et al* ., 2018).**

The endothelin system participates in both physiology and pathology of the cardiovascular system **(Haynes and Webb ,1998)**, although it is important to note that the extent of this contribution is both species- and vascular bed-dependent. The endothelin system has a role in the regulation of basal vascular tone and altered expression/activity of ET-1 could contribute to the development of diseases such as hypertension, atherosclerosis, and vasospasm after subarachnoid hemorrhage , of three endothelin isoforms only ET-1 is produced constitutively by endothelial cells and, therefore, it plays the most important role in regulating vascular function. Interestingly, under inflammatory conditions vascular smooth muscle cells have the capacity to generate ET-1 **(Woods *et al*.,1999).**

A number of stimuli, including thrombin, insulin, cyclosporine, epinephrine, angiotensin II, cortisol, inflammatory mediators, hypoxia and vascular shear stress have been shown to increase ET-1 levels(**Inoue *et al*.,1989)** . Endothelin-1 is secreted mainly on the basal side of the endothelial cells to act on ETA receptors on the underlying smooth muscles cells, as well as on ETB receptors on endothelial and on some smooth muscle cells. The endothelin produced vasodilatation when administered to anaesthetized, spontaneously hypertensive .
This transient depression of blood pressure was explained via the production of NO and prostacyclin by the stimulation of ETB receptors located in endothelial cells . The subsequent vasoconstritive response is mediated by the action of ET-1 on ETA and ETB receptors on vascular smooth muscle cells **(Haynes *et al* .,1995).**

2.8 Enzymes

2.8.1 ATPsynthase

The ATP in a beating heart is used for contraction and associated processes (Ca2l and Na+ transport). Indeed, about 2 % of total cellular ATP is consumed in each heart beat in the rabbits . Clearly, this ATP must be replenished by ATP synthesis. Under physiological conditions, the heart is fully aerobic and over 90% of its ATP is made by mitochondrial oxidative phosphorylation. Thus, to a first approximation, we can consider the heart as a system in which ATP is made by the mitochondrial ATP synthase (F1Fo ATPase) and consumed by contraction. The validity of this model is attested by the correlation between heart work rate and the rate of oxidative phosphorylation (as measured by oxygen uptake) **(David *et al* .,1991)** . In general, however, they have concentrated on the means employed by the cell to modulate the mitochondrial electron transfer chain; the ATP synthase was considered as essentially responding passively to changes in these other events(**Denton ,1990).**

2.8.2 Endothelial (ENOs)

Endothelial NOS is expressed in endothelial cells, the isozyme has also been detected in cardiac myocytes, platelets, certain neurons of the brain, in syncytio-trophoblasts of the human placenta and in LLC-PK1 kidney tubular epithelial cells. Endothelial NOS appears to be a homeostatic regulator of numerous essential cardiovascular functions. Endothelial NOS-derived NO dilates all types of blood vessels by stimulating soluble guanylyl cyclase and increasing cyclic GMP in smooth muscle cells. Deletion of the eNOS gene leads to elevated blood pressure. Nitric oxide released towards the vascular lumen is a potent inhibitor of platelet aggregation and adhesion to the vascular wall. Besides protection from thrombosis,

this also prevents the release of platelet-derived growth factors that stimulate smooth muscle proliferation and its production of matrix molecules. Endothelial NOS is also critical for adaptive vascular remodeling to chronic changes in flow(**Wendy *et al*.,2001).**

There have been some suggestions in recent years that there might be a NOS in or associated with mitochondria. This was originally based on cytochemical and immuno-cytochemical evidence .showing association of either NADPH diaphorase- or NOS antibody-binding to mitochondrial membranes. More recently still there have been reports of NO synthesis within rat liver mitochondrial preparations, and of functional effects of this on respiration**(Ulrich *et al*.,2011).**

CHAPTER THREE

MATERIALS AND METHODS

3. Materials and Methods (3.1.Chemicals)

The materials and methods are summarized in the following table (3-1) with their suppliers of sources.

Table(3-1): Chemicals and kits were used in the study

NO.	Chemicals and Kits	Origin and Company/supply
1	Alpha lipoic acid	United State America (U.S.A)
2	ATP synthase ELIZA Kit	China
3	Cardiac troponin ELIZA Kit	China
4	DMSO	LOBA Chemia
5	Eosine-hematoxylin stain	Merck, Germany
6	Endothelin-1 ELIZA kit	China
7	Ethanol	Merck, Germany
8	Formalin10%	TEDIA company. USA
9	Monosodium glutamate	Japan
10	Nitric Oxide synthase ELIZA KIT	China
11	Nitric oxide ElIZA KIT	China
12	Peroxnitrite ELIZA KIT	China
13	Paraffin wax	Merck, Germany

14	Xylole	Scharlau, Spain

3.2 Instruments

The instrument which were used in this study with their suppliers and sources are shown in table (3-2)

Table(3-2): Instrument with their suppliers:

NO.	Instrument	Suppliers and sours
1	Analytial Sensitive Balance	Sartorius/ Germany
2	Balance for animals	Shimadu company-Japan
3	Centrifuge	Hettich Roto fix11/Japan
4	Digital Camera	Top Cam/China
5	Dissection set	China
6	EDTA tube	Jordan
7	Electric grinder	China
8	ELISA reader	bioKIT/USA
9	ELISA washer	bioKIT/USA
10	Eppendorf tubes	China
11	Freezer	Hitachi/Japen
12	Gel tubes	Jordan
13	Incubator	BINDER/Germany

14	Insulin syringes	Italy
15	Light microscope	Lieca/China
16	Micropipette 100-1000 ul	Germany
17	Micropipette1-100 ul	Germany
18	Mince machine	HITACHI/Japan
19	Optical microscope with a tablet PC	OPTICA/Italy
20	Rotary macrotome	Germany
21	Sterile Syringe(1,3,5and 10 ml)	China
22	Test tubes	China
23	Vet Hematological Auto Analysis	Genex company picture

3.3 Experimental Animals

The study is performed during the period from November,2018 to April 2019.Rabbits kept in animal house for acclimation to the laboratory condition for two weeks. Thirty two healthy adult male rabbits aged (7-9)months ,weighting 1300 -1500 gm. were obtained from the animal house college of science in Baghdad University . They were placed in the animal house of veterinary medicine college of Kerbala University, temperature was maintained at (21-25) C̊ the air of the room was changed continuous by using ventilation vacuum and with light /dark cycle 12: 12 h/day animal fed on the pellet of freshly prepared ration.

3.3.1 The experiment design

Thirty two male rabbits were divided randomly into four groups, (8/group).

1.GI rabbits were given food without supplementation (as control) for 10 weeks

2.GII rabbits were given oral intubation daily 8mg/kg B.W of MSG dissolved by water for 10 weeks according to method reported by(**Rogres and Blundell , 1990)**

3.GIII rabbits were given oral intubation daily 60mg /kg BW of ALA dissolved with DMSO for 10 weeks according to method reported by **(Al-Ali , 2018)**

4.GIV rabbits were given 8mg/kg B.W oral intubation daily of MSG dissolved by water and given 60mg/kg. B.W of ALA . as show in figure (3.1).

3.3.2 Blood collection

Animals were anesthetized by injection of (90mg/kg) Ketamine and (40mg/kg) Xylazine .Blood samples were collected at 10 weeks of study of experiment via heart puncture technique was done by using a 10 ml disposable syringe and 10 ml of blood was drawn slowly and gently . 10 ml of blood collected in gel test tubes (for serum preparation) which leaves for 30 minutes in room temperature and then used for getting serum by centrifugation at 3000 rpm for 15 minutes to separate serum and put in Eppendorf tubes which kept at freezer in -20C . Eppendorf tube which kept at freezer in -20C**(Amin and Ahlfors, 2008)** . At the end of experimental , the rabbits were sacrificed after blood collection by heart puncture technique, the abdominal lumen was opened and heart and aorta were removed and then placed in formalin (10%) as fixative for histological preparation

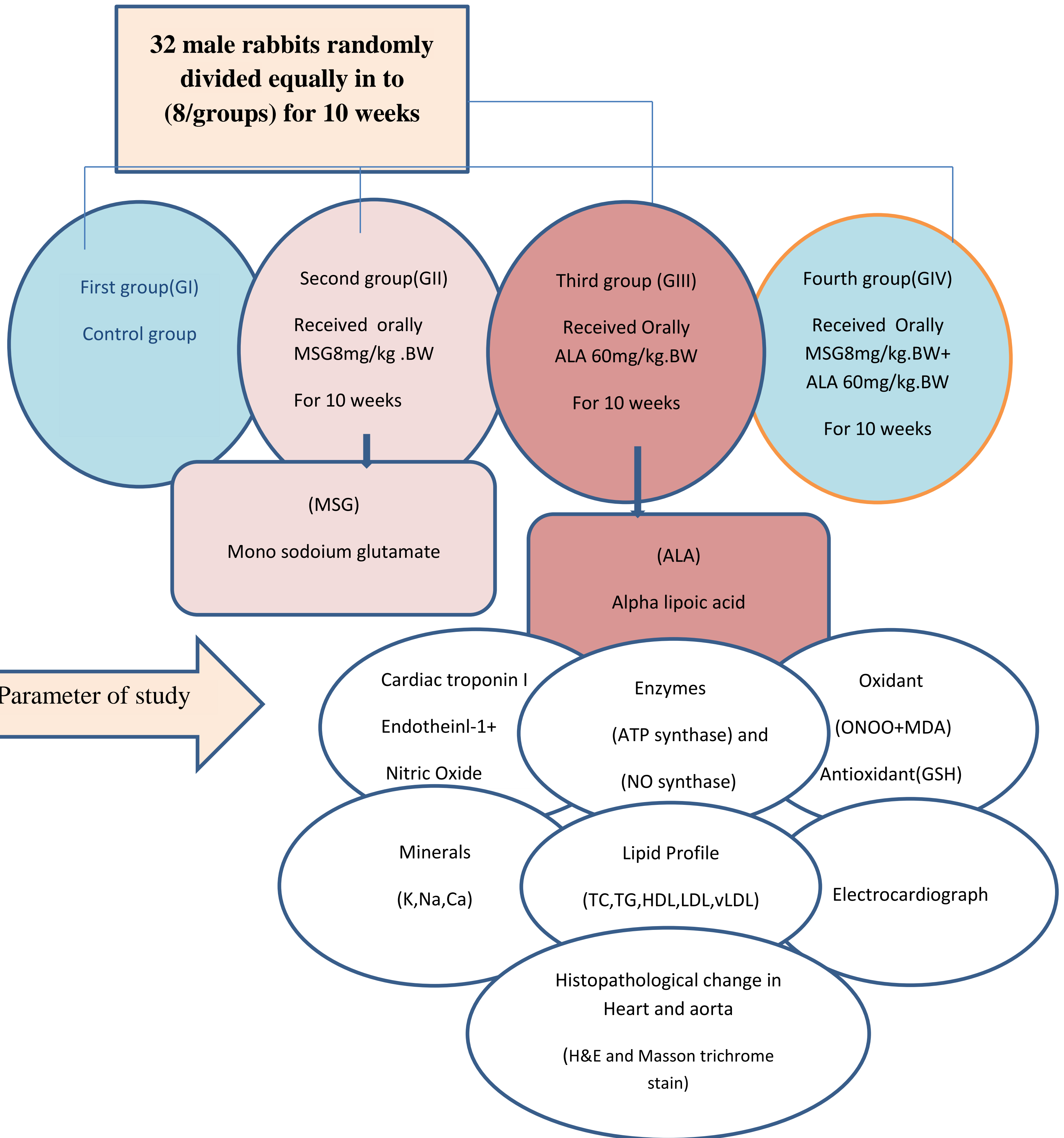


Figure (3.1): Represented experimental design .

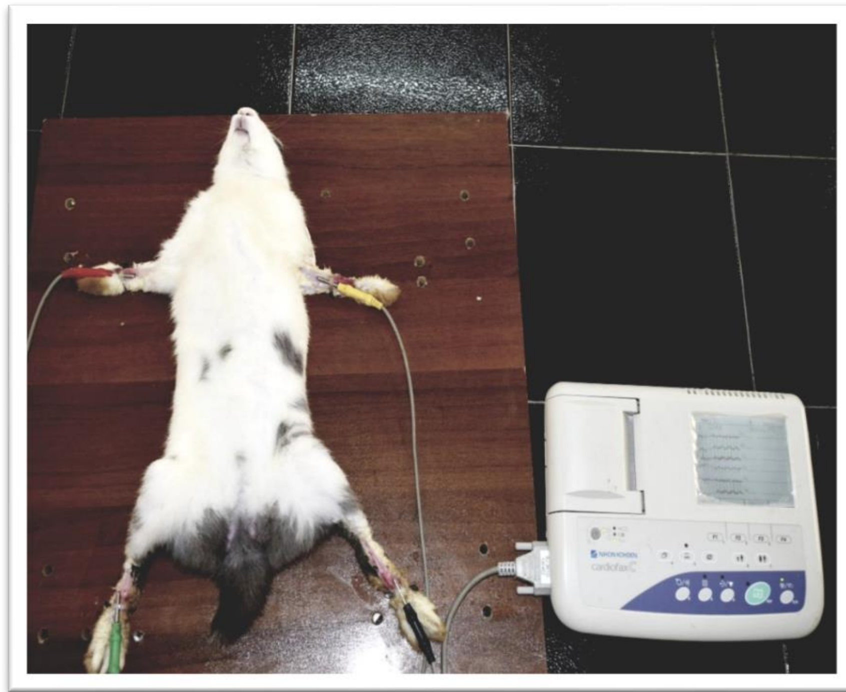
3.4 Electrocardiograph

3.4.1Electrocardiogram recording

The electrocardiogram recoding was carried out using the electrocardiograph after completing the shaving areas in the arms and legs of the hair and after the prepare of the animals on a wooden board equipped for this purpose suitable with the size of animals .Awooden panel pierced from the four sides is linked to the animal and after the special electrodes have been modified to fit with the animals limbs and put the gel material that helps to receive electrical events clearlyas show in figure (3.2) .The electrodes were fixed calmly and accurately after calming the animal repeated the process multiple times before taking readings in order to tame the animal on the device and to keep the animal calm and not to get a state of fear and panic and after the connection leaves the animal for 10 to 15 minute after making sure that the animal was quiet .The measurement was done on the basis of the method of mono-lead which was called (Einthoven Triangle) ,the reading on a method on the amplification force 10Mm/Mv and the electrical voltage 25Mm/s and speed ½ second .

The measurement of the ECG was done without anesthesia , where we note in the diagram of the heart on the special graphic paper which is divided into large squares divided into small squares each large square contains five small squares and be read horizontally and vertically .The vertical –axis voltage of the reading represent the electrical voltage .The small square is equal to(1Mm=0.1Mv) . The horizontal shape (Horizontal axis-time) on the reading. The small square is equal in milliseconds 1Mm=0.04second. The speed of the paper is equal and the p wave represent depolarization in the atria . AS the atria muscle are less thick and smaller than the ventricular muscles ,the device cant capture the electric waves of their

propagation and repolarization and amplify it through the amplifier , the QRS complex wave represent the depolarization of the ventricular muscle .The T wave represent the repolarization of the ventricular muscle which is a state of rest and relaxation.



Figure(3.2) represents how to connect the electrodes of electrical diagram of the male rabbits ,represent the red electrode (right arm) and yellow electrode (left hand) and black electrode (left leg) while green electrode represent ground connector(**Jonathan *et al* .,2009)** .

3.5Biochemical parameter :

3.5.1Estimation of cardiac markers

3.5.1.1 Estimation of serum cardiac troponin I(ng/ml) .

Cardiac troponin I(cTnI) was measured by kit produced by Guangzhou, P.R.China .company **(Adams ,1994) ,**as show Appendix I

3.5.1.2 Estimation of serum Endothein-1 (ng/l)

Endothelin 1 (ET-1) was measured by Rabbit Endothelin 1 Enzyme-Linked Immunosorbent Assay (ELISA) kit by Bioassay Technology Laboratory by method of **(Goldie ,2000)** ,as Shown appendix II

3.5.1.3 Estimation of serum Nitric oxide (NO) µM/L

Nitric oxide (NO) was measured by method of **(Chang *et al* .,1998)** , as Shown appendix III

3.5.2 Estimatiion of serum Enzymes

3.5.2.1 Estimation of serum ATP Synthase (ng/l)

ATP Synthase was measured by Rabbit ATP Synthase Subunit Alpha ,Mitochondrial ELISA Kit by Bioassay Technology Laboratory by method **(Lostcher,1984)** ,as Shown appendix **IV**

3.5.2.2 Estimation of serum NO Synthase (ng/ml)

Endothelial NO Synthase (ENOs) was used by Rabbit Nitric Oxide Synthase 3, Endothelial ELISA Kit by Bioassay Technology Laboratory by method**(Breddt and Snyder , 1994)** , as Shown appendix V .

3.5.3 Estimation of serum oxidant

3.5.3.1 Estimation of serum peroxynitrate (ONOO) µM/L

Peroxynitrate was measured by method of **(Vanuffelen ,1998)** , as Shown appendix VI .

3.5.3.2 Estimation of Serum Malondialdehyde (MDA):µM/l

Malondialdehyhe was estimated by Thiobarbituric acid (TBA) assay method of **(Muslih *et al* .,2002)** , as Shown appendix VII .

3.5.4 Estimation of serum reduced Glutathione (mg/dl)

Serum glutathione concentration has been measured by using the Ellmans reagent method previously used by **(Alzamely *et al*.,2001**) ,as Shown appendix VIII .

3.5.5 Estimation of serum minerals . AS Shown appendix IX.

3.5.5.1 Estimation of serum potassium (K) mmol/l

Specific Kit for measuring Human potassium concentrationin serum was supplied by Egyptian Company for Biotechnology (S.A.E) by method of **(Hoeflmayr,1979).**

3.5.5.2 Estimation of serum sodium (Na) mEq/l

Specific Kit for measuring Human sodium concentrationin serum was supplied by Egyptian Company for Biotechnology (S.A.E) by method of **(Henry *et al* .,1974).**

3.5.5.3 . Estimation of serum calcium (Ca) mEq/l

Specific Kit for measuring Human calcium concentrationin serum was supplied by Biolabo SA . France by method of **(Tietz ,1999).**

3.5.6 Estimation of serum lipid profile , as Shown appendix X

3.5.6.1Estimation of serum total Cholesterol concentration (mg/dL):

Cholesterol concentration was measured by using Cormay cholesterol kit produced by PZ CORMAY S.A. company. after enzymatic hydrolysis and oxidation, the cholesterol is determined in the presence of phenol and peroxidase, the hydrogen peroxide and 4-aminoantipyrine forming quinoneimine the indicator **(Fasce, 1982).**

3.5.6.2Estimation of serum Triglyceride concentration (mg/dL):

Triglyceride concentration was measured by Cormay triglyceride kit produced by PZ CORMAY S.A. company. Its hydrolyzed to glycerol enzymatically according to the following reaction **(Fossati and Prencipe, 1982).**

3.5.6.3Estimation of serum HDL-Cholesterol concentration (mg/dL) :

HDL-Cholesterol concentration was measured by using Cormay HDL kit produced by PZ CORMAY S.A. The supernatant contains high density lipoprotein (HDL). The HDL-cholesterol is then spectrophotometrically measured by means of the coupled reaction described(**Grove, 1979).**

3.5.6.4 Estimation of serum LDL-Cholesterol concentration (mg/dL) :

LDL-C was measured by using Cormay LDL kit produced by PZ CORMAY S.A. company **(Alan, 2006).** As Show appendix (IV).

3.5.6.5 Estimation of serum VLDL-Cholesterol concentration (mg/dL): VLDL-C was measured by using the following equation **(Friedewald, 1972).**

3.6 Histological study

For histopathological study of rabbit were anaesthetized and sacrificed by withdrawal of blood from heart .immediately after death the heart and aorta with its main branches were excised , blotted open longitudinally and preserved in 10% neutral formalin buffer solution till the preparation of histological section several tissue section were prepared according to **(Mescher ,2010).**

3.7 Statistical Analysis

Data was analyzed as one-way ANOVA using the general linear model (GLM) procedure to SPSS 22.0 software **(Corp, 2011).** Four treatment means were separated using a "protected" Duncan`s analysis in level ($p<0.05$).

CHAPTER FOUR

RESULTS

4.Results

4.1 Protective Effect of ALA in proteins cardiac biomarkers (Cardiac troponin I , Endothelin 1, Nitric Oxide) in MSG treated male rabbits

4.1.1 Cardiac troponin I

Table (3.1) illustrated the mean value of serum protein cardiac biomarkers in the control and three treated group along the experimental study .It can be seen that serum cardiac troponin I value to increase significantly value ($p<0.05$) after 10 weeks in MSG treated group as compared to control and another treated (GIII,GIV). On the other hand rabbits received daily intubation of 60mg/kg B.W ALA caused significant ($p<0.05$) decrease in mean value of serum cardiac troponin I in GIII comparing to MSG treated group while combined intubation of ALA with MSG caused significant depletion in this biomarker comparing to other treated groups.

4.1.2 Endothelin-1

The mean value of serum Endothelin 1 concentration in different treated and groups were cleared in table (4.1). the data pointed to the a significantly ($p<0.05$ elevated) in GII after 10 weeks of treatment comparing to control,GIII and GIV . Beside a significantly decrease ($p<0.05$) in the mean value of serum Endothelin-1 were detected after 10weeks of experiment in GIII comparing to GII .

4.1.3 Nitric Oxide

The effect of daily oral intubation of MSG was demonstrated in table (4.1). A significantly ($p<0.05$) decrease in mean value of serum NO concentration was detected after 10 weeks of experiment in GII in comparing to, control , GIII and GIV groups .During the treated period of ALA (after 10 weeks) a significant ($p<0.05$) increase in serum NO concentration were observed in GIII comparing to the control group and other treated groups .

Table (4.1) Effect of daily oral intubation of ALA for 10 weeks on serum (cardiac troponin I , endothelin -1 and NO) concentration of MSG treated male rabbits .

Group / Parameter	GI Control	GII (MSG)	GIII (ALA)	GIV (MSG+ALA)
Cardiac Troponin 1 (ng/ml)	0.00±0.00 C	1.00±0.00 A	0.00±0.00 C	0.33±0.21 B
Endothelin – 1(ng/ml)	10.04±0.25 B	13,16±0.32 A	9.07±0.16 C	10.77±0.13 B
Nitric oxide (µM/l)	30.51±1.91 B	26.19±3.61 C	42.45±3.20 A	29.55±2.33 B

Value are expressed as mean ±SE n=8/group , significant differences ($p<0.05$) ,.The different letters refer to significant difference between difference groups . GII (Monosodium glutamate) ,GIII (Alpha lipoic acid), GIV received (Alpha lipoic acid +Monosodium glutamate) .MSG=8mg/kg B.W; ALA=60mg/kg B.W.

4.2 Protective Effect of ALA in enzyme activities (ATP synthase +Nitric Oxide synthase) in MSG treated male rabbits

4.2.1.ATP synthase

Table (4.2) illustrated the mean value of ATP synthase activity in male rabbit after oral intubation of MSG for 10 weeks which pointed to a significant ($p<0.05$) increment in this parameter was shown at the end of experiment in group GII comparing to control ,GIII and GIV groups .Intubation of ALA treated rabbits GIII caused significant ($p<0.05$) decrement in serum ATP synthase comparing to control and GII groups .There was non-significant ($p<0.05$)in serum ATP synthase combined intubation of ALA and MSG treated rabbits .

4.2.2.Nitric Oxide synthase

The effect of daily oral intubation of MSG on mean value of serum NO synthase activity , was shown a significant reduction ($p<0.05$) after 10 weeks as compared with GI,GIII,GIV groups .

A highest significant increase ($p<0.05$) in this parameter was appeared in rabbit in GIII group comparing to control GII and GIV groups . The group treated with (MSG +ALA) showed significant reduction ($p<0.05$) in the activity of NO synthase in the comparison with GII, GIII, as shown in table (4.2).

Table (4.2) Effect of daily oral intubation of ALA for 10 weeks on serum enzymes (ATP synthase , NO synthase) activities of MSG treated male rabbits .

Group Parameter	GI Control	GII (MSG)	GIII (ALA)	GIV (MSG+ALA)
ATP synthase(ng/l)	133.61±2.10 B	170.48±4.19 A	112.46±3.15 C	123.98±2.17 B
Nitric Oxide synthase (ng/ml)	0.24±0.004 B	0.21±0.001 C	0.29±0.007 A	0.23±0.002 B

Value are expressed as mean ±SE n=8/group , significant differences ($p<0.05$) ,.The different letters refer to significant difference between difference groups . GII (Monosodium glutamate) ,GIII (Alpha lipoic acid), GIV received (Alpha lipoic acid +Monosodium glutamate) .MSG=8mg/kg B.W; ALA=60mg/kg B.W.

4.3 Protective role of ALA in oxidant (ONOO,MDA) concentration in MSG treated male rabbits and serum antioxidant (GSH).

4.3.1.ONOO(Peroxynitrate)

The effect of daily oral intubation of MSG for 10 weeks caused significant ($p<0.05$) increase in the mean value of ONOO concentration in GII in comparing to the control GI,GIII, and GIV treated group .It appears also that ALA intubation caused reduction in mean value of ONOO after 10 weeks of experiment in compared with GI , as shown in in table (4.3).

4.3.2.MDA(Malondialdehyde)

The table(4.3) showed a general trend for the MDA value to increase significantly ($p<0.0$) after 10weeks in MSG treated groups (8mg/kg B.W) GII as compared to control group and other treated groups(GI,GIII,GIV) . The result also showed that oral intubation of (60mg/kg B.W) of ALA caused significant ($p<0.05$)decrease in serum MDA concentration of mean value in comparing to GI GII and another groups, on the other hand combined intubation of MSG with ALA caused non significant in this parameter comparing to GII,GIII groups .

4.3.3.GSH(Glutathione)

Table (4.3)showed the mean value of serum GSH concentration a significantly decrease ($p <0.05$) after ten weeks in MSG treated GII (8mg/kg B.W) GII as compared to GIII and GIV groups .The result also showed that oral intubation of (60mg/kg B.W) of ALA caused significant ($p<0.05$)increase in serum GSH comparing to GII .

Table (4.3) Effect of daily oral intubation of ALA for 10 weeks on serum oxidant (MDA and ONOO) and antioxidant (GSH) concentration of MSG treated male rabbits .

Group / Parameter	GI Control	GII (MSG)	GIII (ALA)	GIV (MSG+ALA)
ONOO (µM/l)	4.05±0.83 B	6.44±0.39 A	3.20±0.69 C	4.008±0.59 B
MDA(µM/l)	0.012±0.001 AB	0.017±0.002 A	0.007±0.0009 C	0.014±0.001 AB
GSH(mg/dl)	9.82±1.98 BC	5.70±1.21 C	17.76±2.68 A	13.49±2.47 AB

Value are expressed as mean ±SE n=8/group , significant differences ($p<0.05$) ,.The different letters refer to significant difference between difference groups . GII (Monosodium glutamate) ,GIII (Alpha lipoic acid), GIV received (Alpha lipoic acid +Monosodium glutamate) .MSG=8mg/kgB.W; ALA=60mg/kgB.W.

4.4 Protective role of ALA in Body weight and heart weight in MSG treated male rabbits.

4.4.1 Body weight

A highest significant increase ($p<0.05$) in body weight appeared in rabbits received (8mg/kg B.W)of MSG daily for 10 weeks in comparison to control group and another treated groups(GIII,GIV). A significant($p<0.05$) decrease in mean value of BW concentration was detected after 10 weeks of experiment in GIII received ALA in compared with control group , as shown in table (4.4)

4.4.2 Heart weight

The table (4.4) showed the effect of oral intubation of MSG trend for the heart weight value to increase significantly ($p<0.05$) after 10 weeks in GII as comparing to control group and another treated groups (GIII,GIV) .

The result also showed that oral intubation of ALA caused significant ($p<0.05$) decrease in heart weight in GIII comparing to control group and GIV.

Table (4.4) Effect of daily oral intubation of ALA for 10 weeks on body and heart weight of MSG treated male rabbits.

Group Parameter	GI Control	GII (MSG)	GIII (ALA)	GIV (ALA+MSG)
Body Weight(gm/kg)	1285.83±15.62 C	1715.00±42.32 A	1095.33±31.14 D	1415.00±12.31 B
Heart Weight(g/kg)	8.63±0.06 B	12.43±0.32 A	7.50±0.13 C	8.69±0.10 B

Value are expressed as mean ±SE n=8/group , significant differences (p<0.05) ,.The different letters refer to significant difference between difference groups . GII (Monosodium glutamate) ,GIII (Alpha lipoic acid), GIVreceived (Alpha lipoic acid +Monosodium glutamate) .MSG=8mg/kg B.W; ALA=60mg/kg B.W.

4.5 Protective role of ALA in serum lipid profile in MSG treated male rabbits

The effect of daily oral intubation of MSG and ALA on mean value of serum lipid profile concentration were demonstrated in table (4.5) . A significantly ($p<0.05$) increase in mean value of serum TC ,TG ,LDL-C,VIDI-C concentration were detected after 10 weeks of experiment in GII in comparing to control group and another treatmentd groups (GIII,GIV).and significant ($p<0.05$) decrease in mean value of serum HDL concentration was detected after 10 weeks of experiment in GII comparing to ALA group which showed significant ($p<0.05$) increase after 10 weeks of experiments comparing to (GI ,GII,GIV)groups .On the other hand ,rabbit received only ALA showed a significant reduction ($p<0.05$) in mean value of serum bad lipid profile comparing to GII group .

Table (4.5) Effect of daily oral intubation of 60mg/kg B.W of ALA for 10 weeks on serum lipid profile concentration in 8mg/kg B.W of MSG treated male rabbits.

Group / Parameter	GI Control	GII (MSG)	GIII (ALA)	GIV (MSG+ALA)
Cholesterol(mg/dL)	2.20±0.43 B	3.18±0.21 A	0.78±0.15 C	1.48±0.13 BC
Triglyceride(mg/dL)	0.63±0.13 B	2.20±0.66 A	0.35±0.0.04 C	0.51±0.17 B
HDL(mg/dL)	1.45±0.15 B	0.21±0.04 C	1.96±0.10 A	1.41±0.04 B
LDL(mg/dL)	35.61±2.05 B	82,03±13.21 A	19.35±2.14 C	33.33±2.62 B
VLDL(mg/dL)	8.58±0.20 B	14.17±1.52 A	5.14±0.46 C	8.05±0.61 B

Value are expressed as mean ±SE n=8/group , significant differences (p<0.05) ,.The different letters refer to significant difference between difference groups . GII (Monosodium glutamate) ,GIII (Alpha lipoic acid), GIV received (Alpha lipoic acid +Monosodium glutamate) .MSG=8mg/kg B.W; ALA=60mg/kg B.W.

4.6 Protective role of ALA in serum (K, Na,Ca) concentration in MSG treated male rabbits.

4.6.1.Potassium

A significant ($p<0.05$) decrease in mean value of serum [K] concentration was detected after 10 weeks of experiment in groups GII treated with MSG comparing to control and GIII groups ,result also showed that oral intubation of ALA caused significant ($p<0.05$) increment in mean value of serum[K] concentration of ALA treated rabbits (GIII) comparing to MSG treated group and control group ,as shown in (table 4.6).

4.6.2.Sodium

Depending on the result clarified in table (4.6) , there were a significant($p<0.05$) elevation in the serum [Na]concentration in GII received MSG comparing to groups ,GI and GIII. In the 10 weeks of the experiment ,a significant decrease($p<0.05$) in serum [Na] was observed concentration in GIII received ALA comparing to GII group .

4.6.3.Calcium

The effect of daily oral intubation of MSG non-significant ($p<0.05$) in mean value of serum [Ca] concentration was detected after 10 weeks , in GII comparing to G1,GIII ,GIV groups . as show in (table 4.6) .

Table (4.6) Effect of daily oral intubation of ALA for 10 weeks on serum[K, Na and Ca] concentration of MSG treated male rabbits.

Group / Parameter	GI Control	GII (MSG)	GIII (ALA)	GIV (MSG+ALA)
K (mmol/l)	4.41±0.34 A	3.10±0.35 B	5.41±0.69 A	5.15±0.46 A
Na(mEq/l)	135.01±1.47 B	150.35±4.14 A	125.86±5.13 B	130.17±6.28 B
Ca(mEq/l)	2.26±0.05 A	2.31±0.07 A	2.20±0.02 A	2.30±0.07 A

Value are expressed as mean ±SE n=8/group , significant differences ($p<0.05$) ,.The different letters refer to significant difference between difference groups . GII (Monosodium glutamate) ,GIII (Alpha lipoic acid), GIV received (Alpha lipoic acid + monosodium glutamate) .MSG=8mg/kgB.W; ALA=60mg/kgB.W.

4.7 Protective role of ALA in Electrocardiograph (ECG) waves in MSG treated male rabbits.

There was no significant ($P<0.05$) difference in wave (P,QRS, T) in the mean value of lead II ,between experimental groups (GI,GIV) as shown in table (table 4.7).

There was a significant increase ($p<0.05$) in the mean value of waves (P,QRS,T) in the lead II was shown in the end of the experiment and detected after 10 weeks in GII received MSG compare to GIII received ALA and GIV while combined intubation of MSG and ALA treated rabbits and caused significant decrease ($p<0.05$) in compared to GII. Also the same table showed A significant decrease ($p<0.05$)in the mean value of HR in the GII compared to another groups (GI,GIII,GIV).and shown a significant increase ($p<0.05$) in mean value of (ST ,QT) intervals in the GII compared to another groups(GI,GIII and GIV) .

Table (4.7) Effect of daily oral intubation of ALA for 10 weeks on Electrocardiograph (ECG) waves of MSG treated male rabbits.

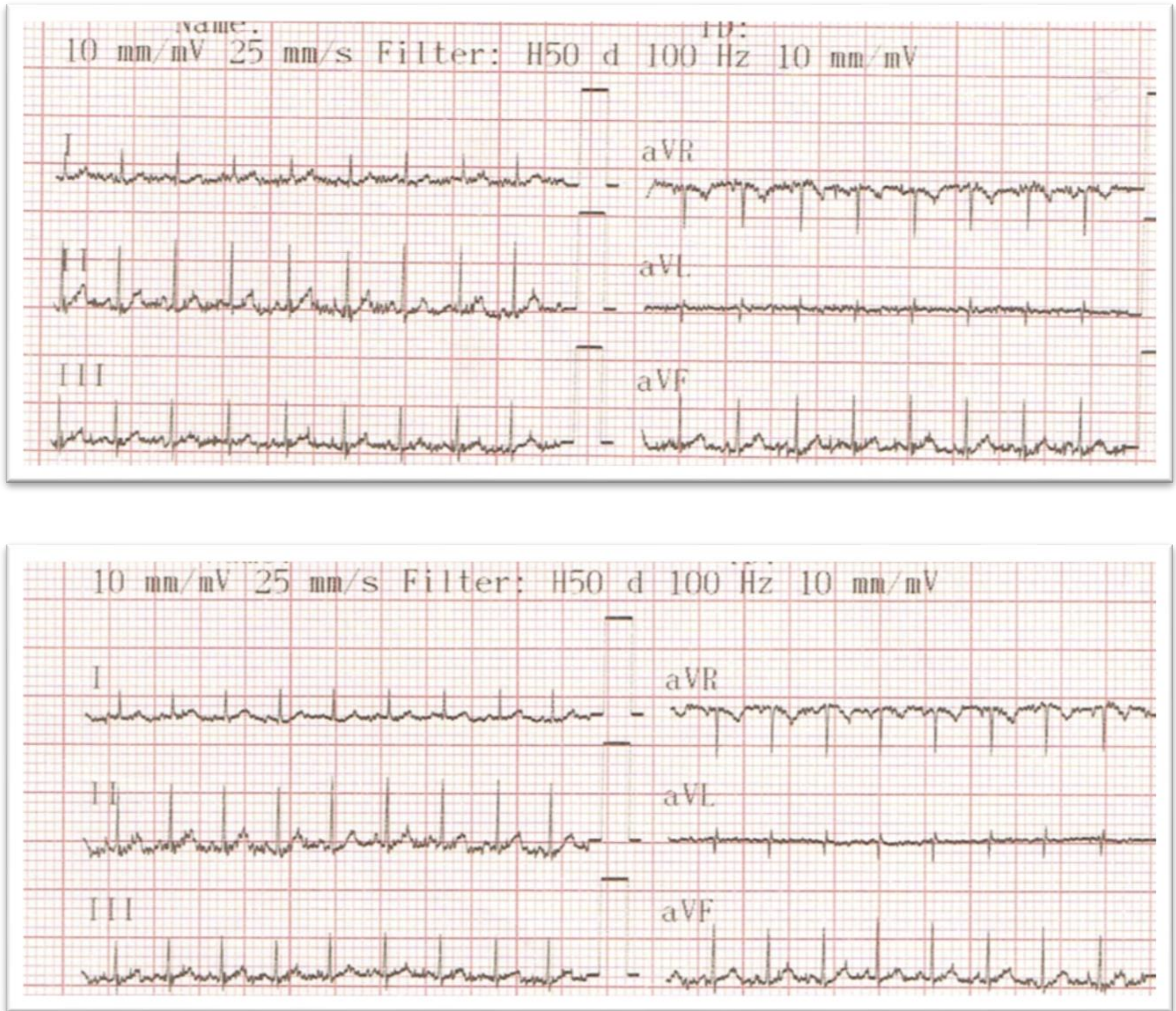
Group / Wave	GI control	GII (MSG)	GIII (ALA)	GIV (ALA+MSG)
QRS (s)	4.16±0.16 BC	5.50±0.56 A	4.00±0.25 C	5.00±0.00 AB
QRS (mv)	0.02±0.003 B	0.04±0.00 A	0.02±0.002 B	0.03±0.002 A
P (s)	1.16±0.16 B	1.33±0.21 A	1.16±0.16 B	1.14±0.00 B
P(mv)	0.02±0.003 B	0.04±0.003 A	0.02±0.004 B	0.03±0.002 B
T(s)	2.66±0.21 AB	3.00±0.36 A	2.16±0.16 B	2.00±0.00 B
T (mv)	0.07±0.002 B	0.09±0.008 A	0.07±0.004 B	0.05±0.002 C
HR (b/m)	250.00±2.58 A	208.17±10.52 B	253.00±7.63 A	255.33±10.54 A
ST (s)	0.11±0.002 B	0.13±0.002 A	0.10±0.002 C	0.11±0.002 B
QT(s)	0.118±0.001 B	0.148±0.003 A	0.117±0.001 B	0.116±0.001 B

Value are expressed as mean ±SE n=8/group , significant differences ($p<0.05$) ,.The different letters refer to significant difference between difference groups . GII (Monosodium glutamate) ,GIII (Alpha lipoic acid), GIV received (Alpha lipoic acid +Monosodium glutamate) .MSG=8mg/kgB.W; ALA=60mg/kgB.W.

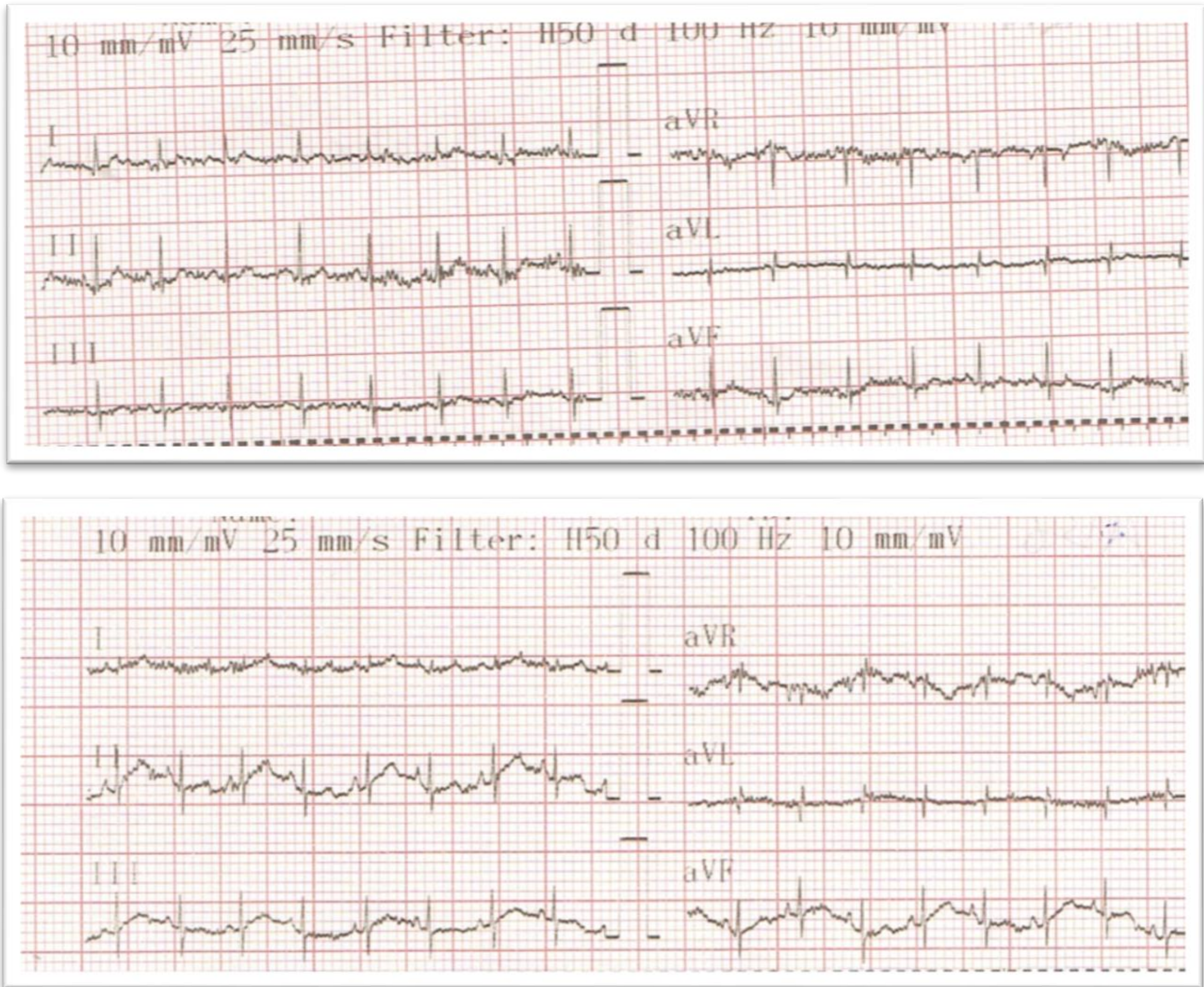
4.7.1.Electrocardiograph intervals of the male rabbits

Electrocardiograph intervals of GI(Control) showed non significant differences in GI in male rabbits . The result of the distance between R-R interval equal 0.24milliseconds ,the length the period between the systolic ventricular and diastolic which represent the QT interval equals 0.12 milliseconds .the ratio of the P-R interval represented the time required for the electrical signal interval from the atrium to SA node is equal to the 0.08 millisecond .

The value of the electrical wave QRS complex represented the ventricular depolarization equals 0.06 millisecond as show in figure (4.1). Electrocardiograph of GII received MSG 8mg/kg B.W in male rabbits ,showed significant prolongation of (P, QRS ,T), alter of ST,QT intervals showed in figure (4.2) and decease in Heart rate comparing with other group (GI,GIII,GIV) and Electrocardiograph of GIII received ALA 60mg/kg B.W ,Show non significant prolongation of waves (P,QRS,T) in GIII and non significant in HR comparing with GII as show in figure (4.3). Electrocardiograph of GIV received MSG8mg/kg B.W and ALA 60mg/kg B.W, showed no significant change in prolongation waves (P,QRS,T) and no significant alteration in (ST, QT) intervals and without decrease in HR in GIV comparing with GII as show in figure (4.4) .



Figure(4.1): Electrocardiograms of GI (control) without any supplementation in male rabbits .



Figure(4.2) Electrocardiograms of GII received MSG 8mg/kg B.W in male rabbits .

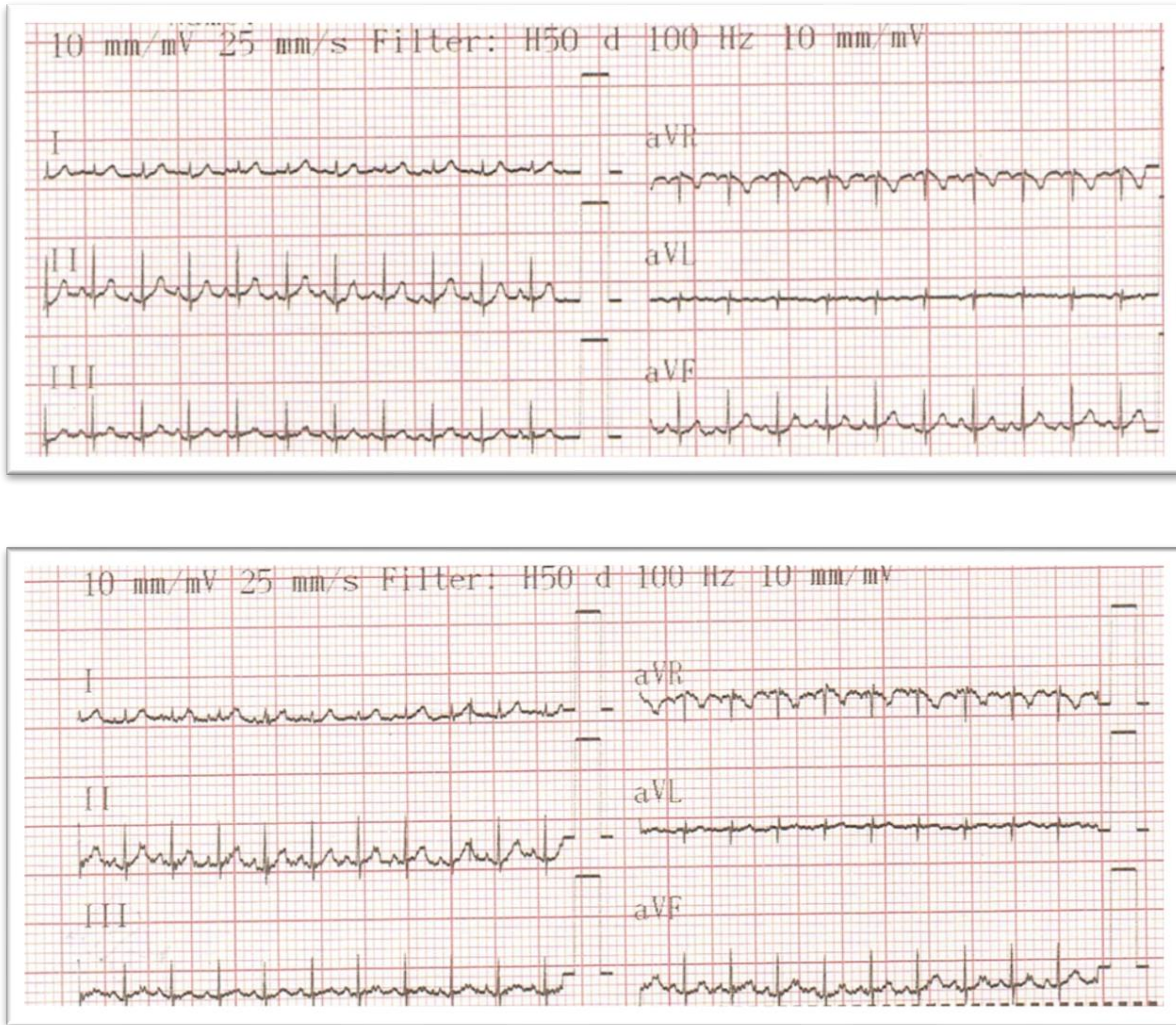
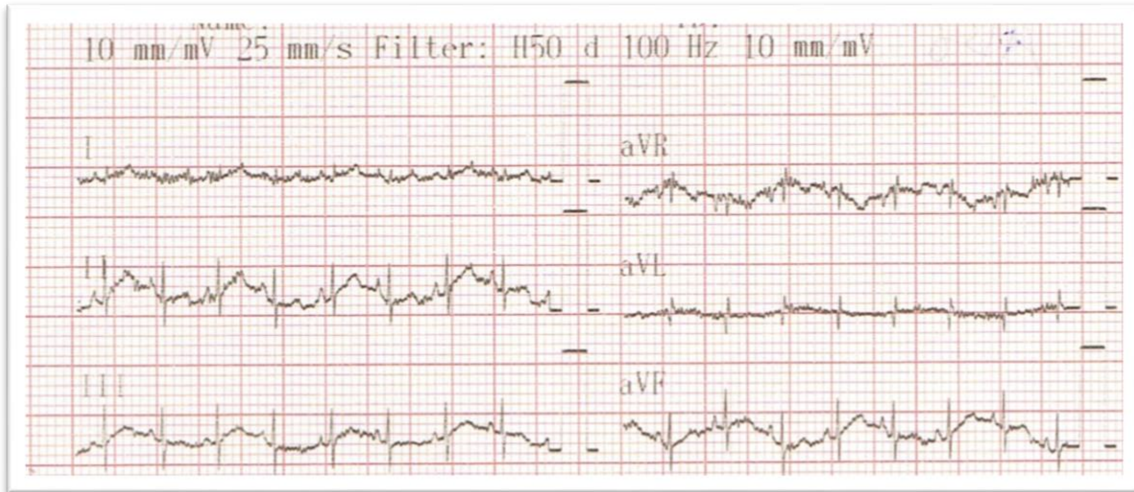
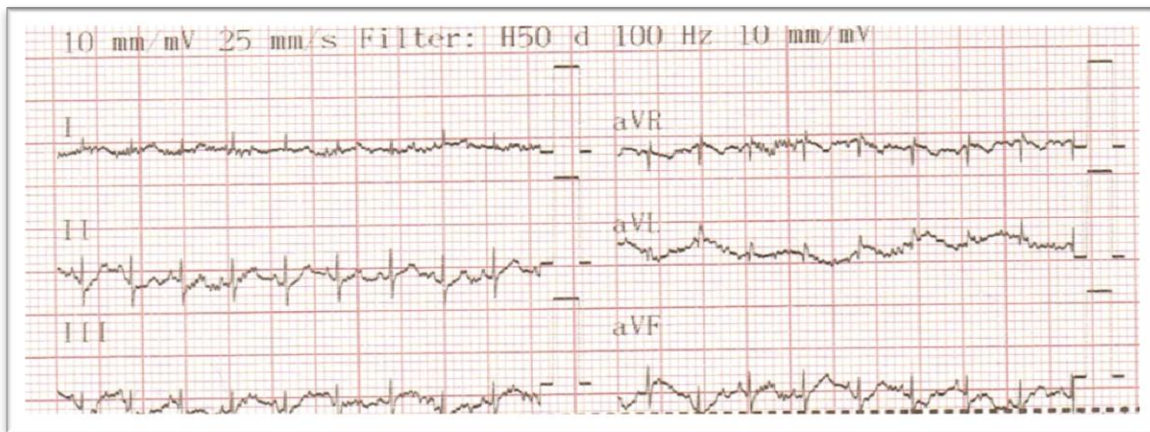


Figure (4.3) : Electrocardiograms of GIII recessived (ALA) 60mg/kg B.W in male rabbits.





Figure(4.4) : Electrocardiograph received (MSG)8mg/kg B.W and (ALA) 60mg/kg B.W in male rabbits

4.8 Histopathological study

4.8.1 Histomicrometry examination

All histological stains used in control group of our study .It appears the structure of elastic artery as consists of three layers, tunic intima which contain endothelium, basal lamina, and sub endothelial connective tissue, smooth muscle cells, internal elastic lamina that represent first layer.The tunic media was composed from greater fenestrated elastic laminae with lesser amount of smooth muscle with collagen fibers second layer thicker evaluated (588.4±52.8), fig.(4.5) table(1) .The third layer connective tissue concentrate collagen with scatter of elastic fibers and vaso vasorum this structure was named tunic adventitia fig.(4.6,A,B).

histomorphological study in our research of the aortic artery in supplementation rabbits group with MSG revealed the nuclei of endothelial cells in intimal surface as irregular and lost squamous cells specialty with foam cell existences and increased aortic medial thickness measured (1054.3±39.9),fig.(4.6,C,D,3.C,D)table(1) and deterioration architectural of elastic fibers in tunica media when compared with control rabbit groups figs.(4.6,A,B, 3A,B) in addition greater of adipocytes penetrated connective tissue of adventitial layer fig.(4.8, A,B) .

Microscopic section examined of the MSG &ALA supplementation rabbits group that shows histomorphological architectural near from the control groups.The endothelial cells of intimal layer squamous characteristic ,so that when we image analysis observed, decreased aortic media layer thickness, was (660.12 ±81),fig.(4.5) table (1) compared with the treated MSG rabbits group and appear

more regulars', +more organized structure of lamina fibers and reduced proliferate smooth muscles in tunica media as in figs. (4.6, E, F; 3, G, H).

Our outcomes of research ,showed three layers of the aorta in ALA.The treated rabbit groups (intima, media and serosa) had normal microscopical details structures the decreased thickened in aortic media evaluated was (615.8 ± 77.5) fig (4.5) table(1) when comperes with MSG treated rabbits group these revealed micrograph (4.6,G,H;3,C,D).

The basic histological structures of the wall heart was composed from three layers, outer epicardium, myocardium and endocardium.The middle was thickest heart layer ,that consists of cardiac branches attune and striated muscles with central nucleus figure (4.9), loose connective tissue with sheet of mesothelium cells formed epicardium, figure (4.9).

The cardiac histomicrometry section of rabbits group treated with MSG revealed a focal area of coagulated necrosis of the myocardium, endocardium edema, fibers separation cloudy, mononuclear inflammatory cells infiltration and loss of striation in myofibers, also, vascular congestion can be observed as in figure (4.10).

The results of our research demonstrated that MSG and ALA supplementation for 10 weeks markedly alleviated the damage MSG disorientation in cardiac tissue, by improving histological picture of heart layers and return striation in myofibers, less and absent blood vessel congestion, edema and cloudy figure (4.11). The histological section specimens of rabbit groups' administration with ALA showed the normal structure of cardio- morphology, figure (4.12)

Table (4.8) Effect of daily oral intubation of ALA for 10 weeks on thickness tunica media of aortic of MSG treated rabbits.

Group	Control GI	GII(MSG)	GIII(ALA)	GIV(MSG+ALA)
Tunica media	588.4±52.8 A	1054.3±39.9 B	615.8 ± 77.5 A	660.12 ±81 A

Value are expressed as mean ±SE n=8/group , significant differences (p<0.05) ,.The different letters refer to significant difference between difference groups . G2 (Monosodium glutamate) ,G3 (Alpha lipoic acid), G4recessive (Alpha lipoic acid +Monosodium glutamate) .MSG=8mg/kgB.W; ALA=60mg/kgB.W.

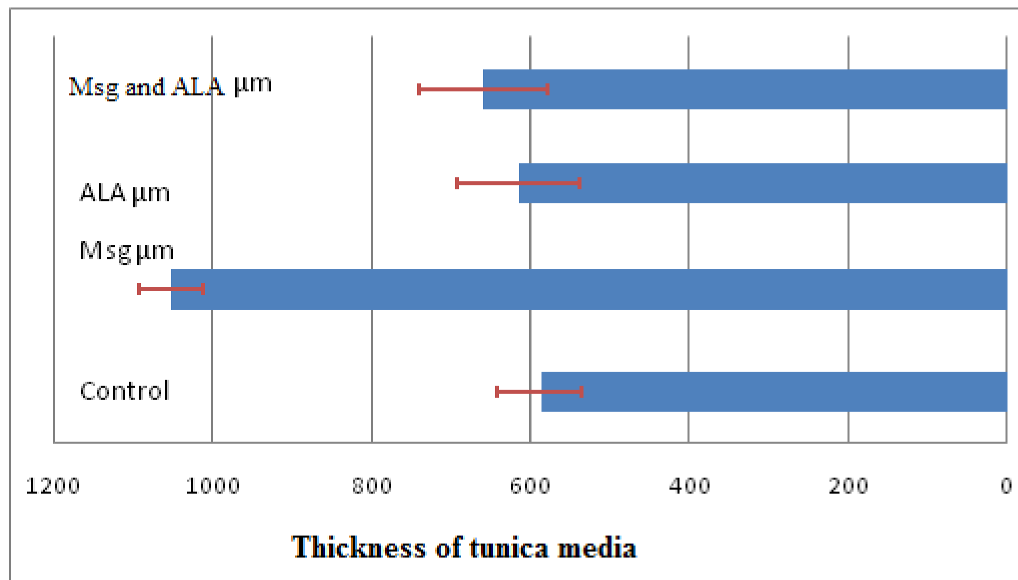


Figure (4.5): The thickness of tunica media of aortic artery in both control and treated male rabbits group. Values represent means±SD. Different letters means significantly (P<0.05) different. Where, control male group **(C.);** monosodium glutamate treated male group **(Msg);** alpha lipoic acid treated male group **(ALA);** companied monosodium glutamate and alpha lipoic acid treated male group **(Msg &ALA).**

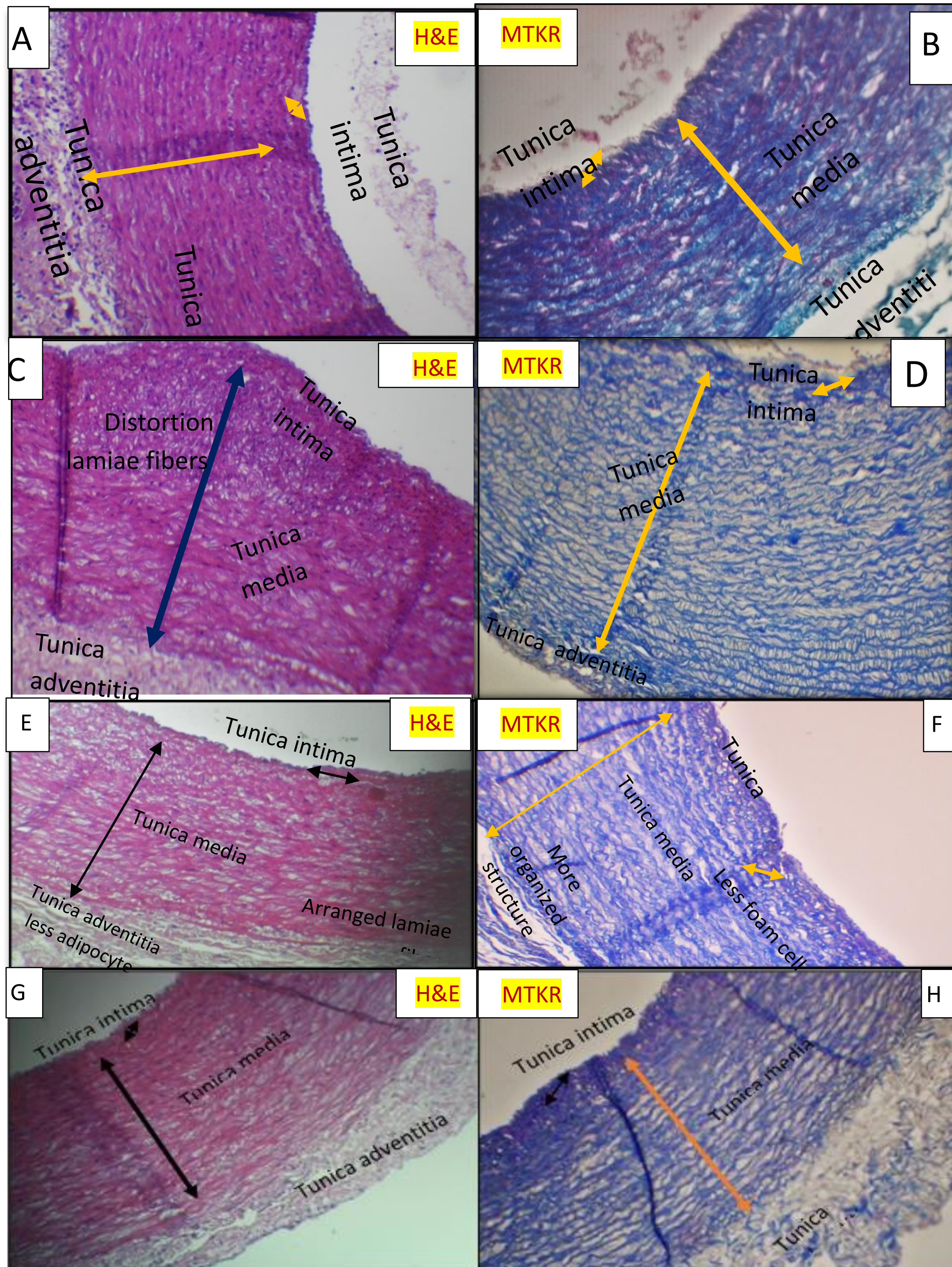


Figure (4.6) : photomicrograph of aortic artery in rabbits: A-B, it look like normal, **C-D.** Belonging to the MSG treated rabbits group revealed thickened in aortic media with disarranged lamenae fibers in addition endothelial cells lost squamous structure, **E-F.** Belonging to the MSG & ALA treated rabbits group, which shows return the normal histological organized, **G-H.** Belonging to the ALA treated rabbits group, that appear assembly normal structure, and Stained with H&E**, A, C, E&G** stained with MTKR **B, D,F&H**. **Magnification: A, B,C,D,E& H** 10X

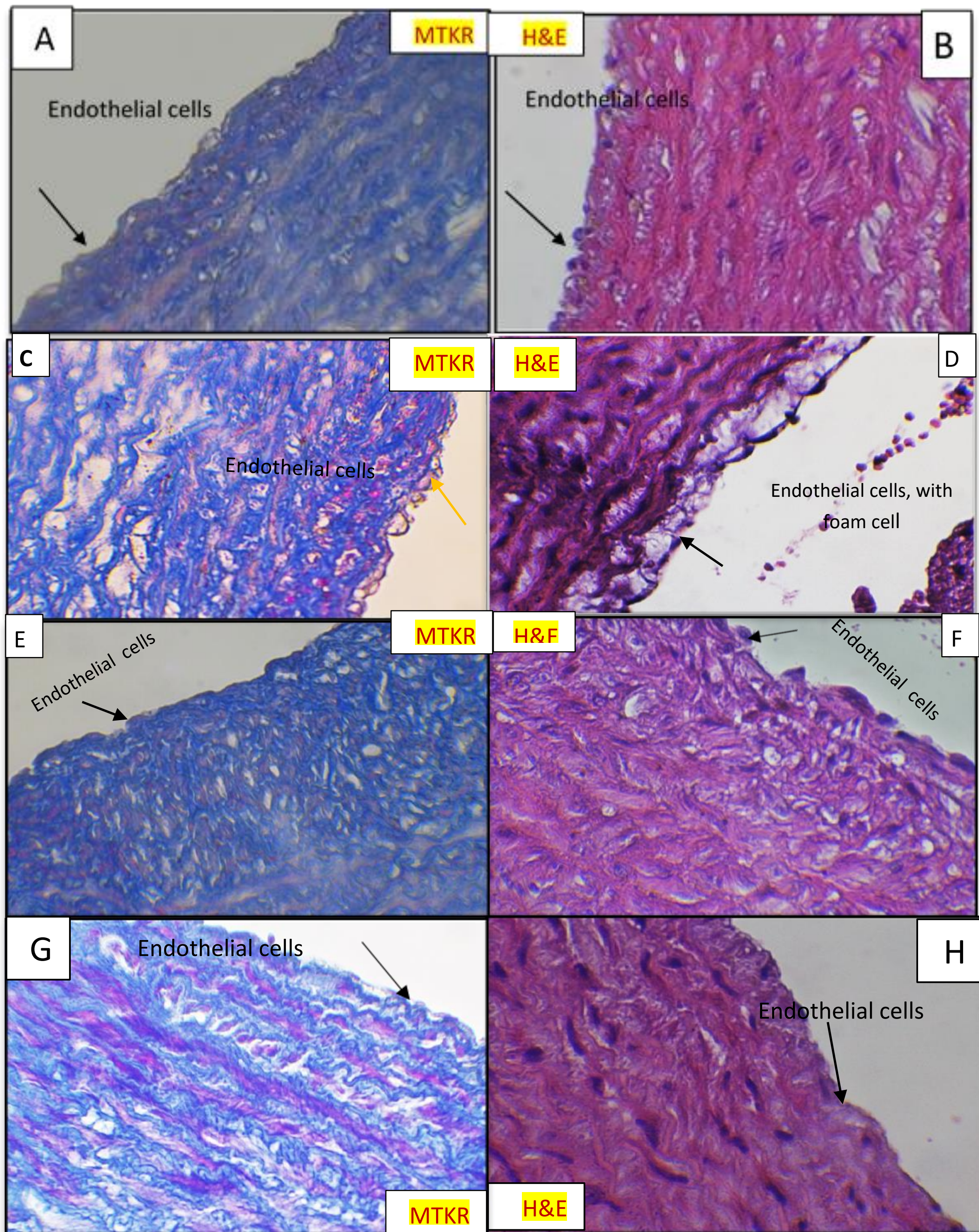


Figure (4.7): photomicrograph of aortic artery in rabbits A-B., it look like normal **C-D.** Belonging to the MSG treated rabbits group revealed nucleus abnormal poison so endothelial cells lost squamous structure, with foam cell, **E-F.** Belonging to the MSG & ALA treated rabbits group, which shows return the normal histological organized, **G-H**. Belonging to the ALA treated rabbits group that appear assembly control structure. Stained with MTKR: **A, C, E&G** stained with H&E: **B, D,F&H. Magnification: A, B, C, D, E& H** 40X

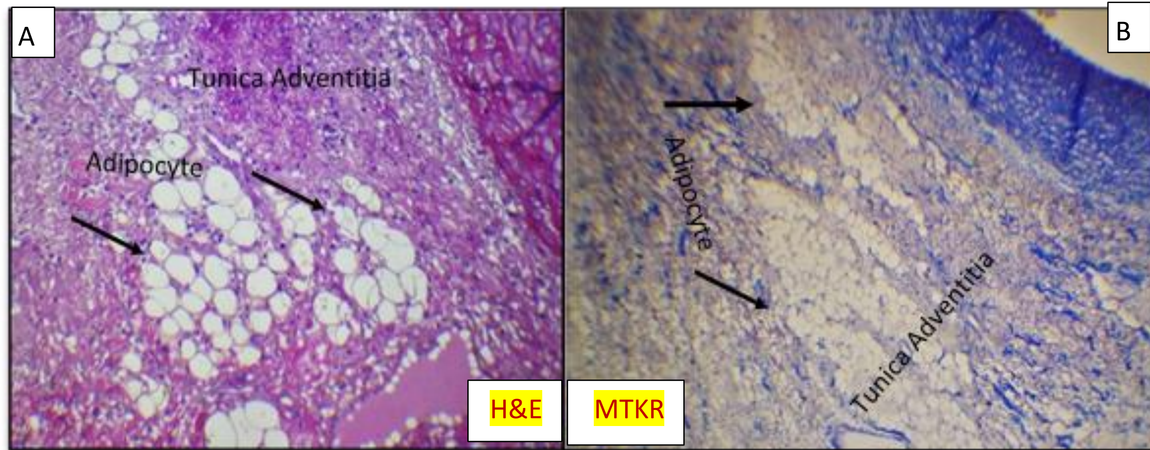


Figure (4.8): photomicrograph of aortic artery in rabbits A-B.Sections belonging to the MSG supplementation rabbits group, shows the invaded fat tissue in the tunica adventitia, and Stained with H&E, **A.** Stained with MTKR **B,**. **Magnification: A, B 4**X

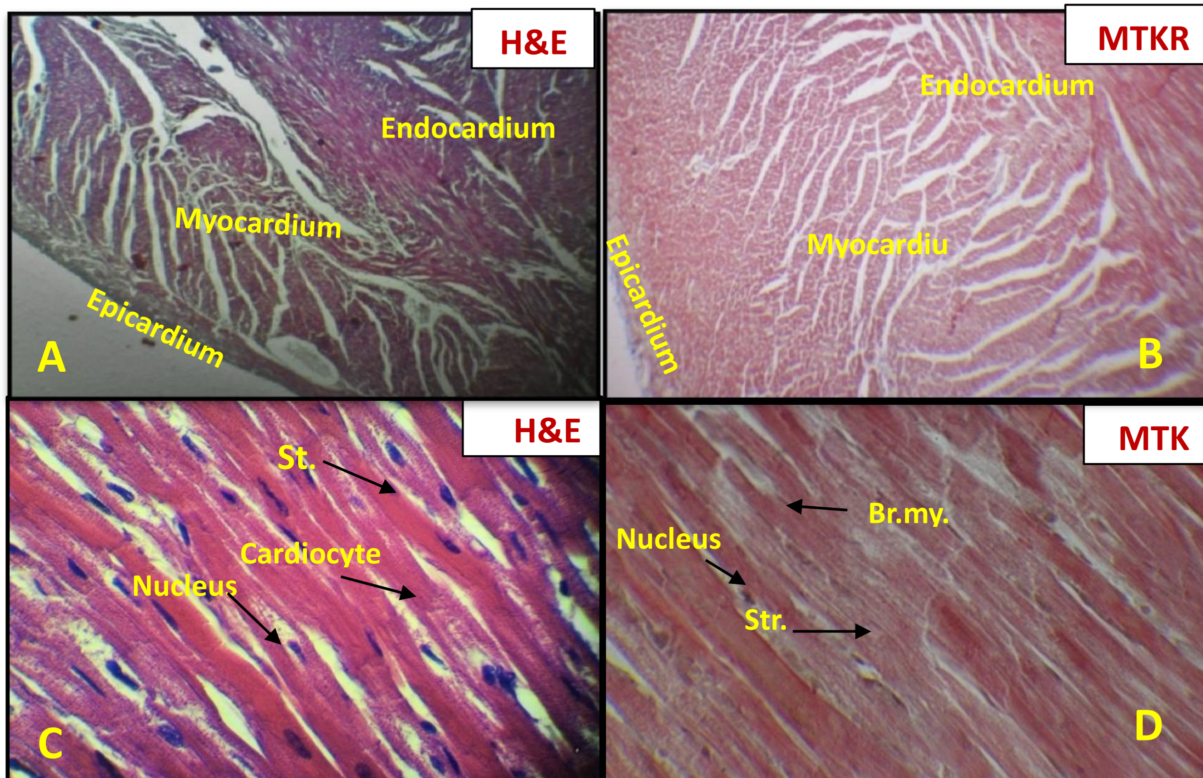


Figure (4.9): photomicrograph of heart in rabbits, A-D. Sections belonging to the control rabbits group, which shows the normal histological organized, epicardium, myocardium which is thicker layer composed from branch, striated cardiocyte with central nucleus, and endocardium. Stained with H&E**, A, C,** and stained with MTKR **B, D, Magnification: A, B 1**0X, **C, D** 40X.

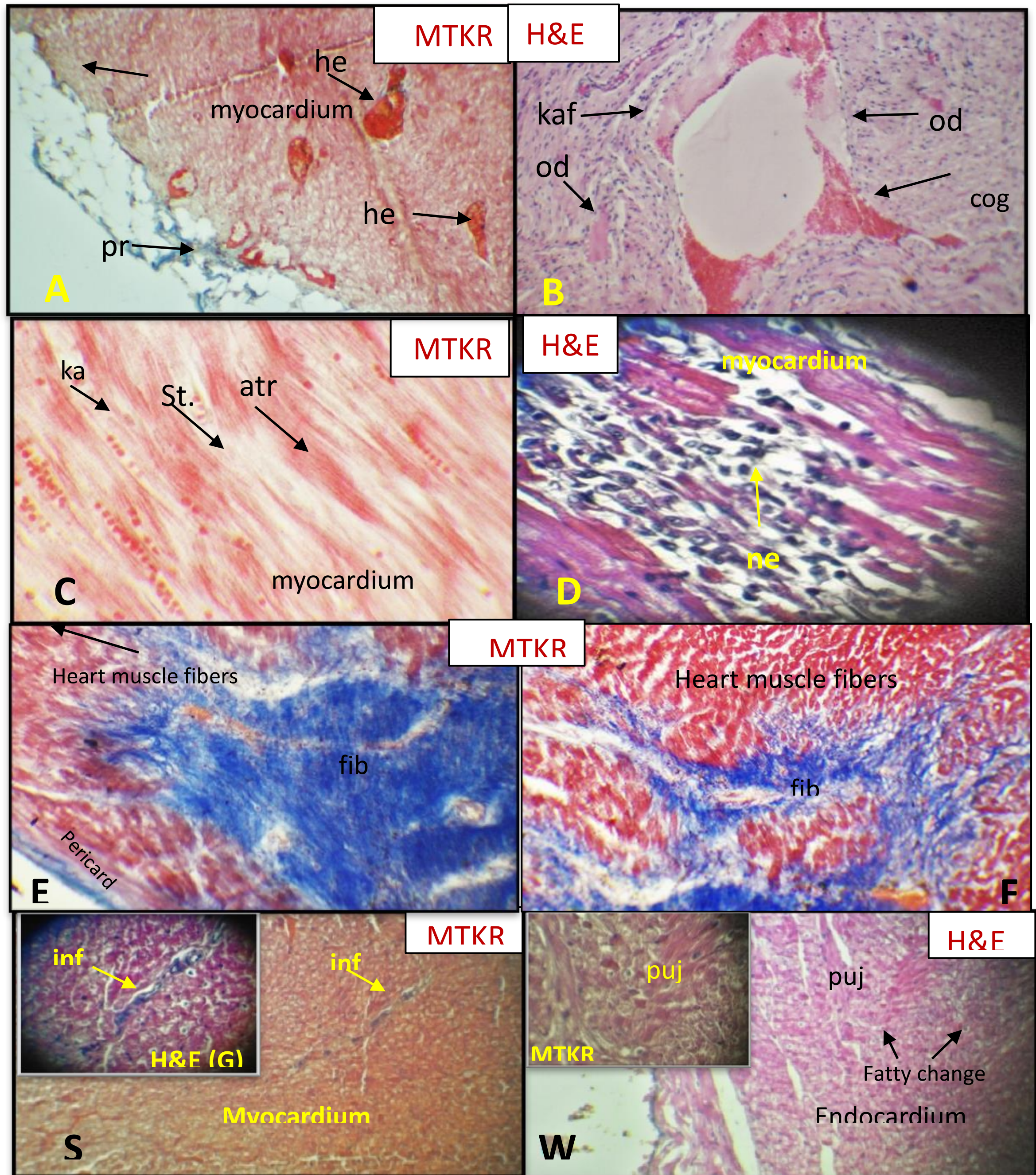


Figure (4.10): photomicrograph of heart in rabbits: **A-W.** Sections belonging to the MSG treated rabbits group revealed epicardium (pr.)and myocardium slight odema (od), hemorrhage(he), congested (cog), and necrosis(ne), absent striation with karyolysis and different type infiltration of inflammatory cells (monocyte) (kaf) in addition disarranged and atrophied muscular fibers (atr) , blue area represented sever fibrosis parts of myocardium (fib.), pyknotic nucleus purkinje, fatty change (puj) and (pavementing) different type infiltration cells and formed inflammatory zone (inf). Stained with H&E**, B, D, W &G,** stained with MTKR **A, C, E, F, S &H. Magnification: A, B, E ,F, S &W** 10X,,**C, D, G & H,** 40X.

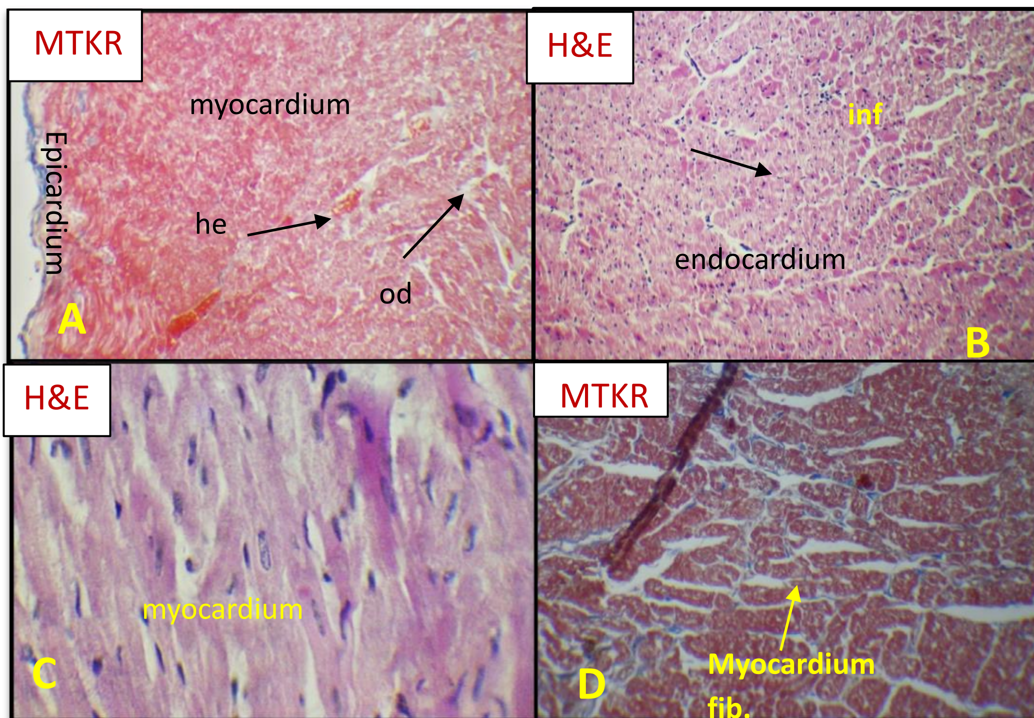


Figure (4.11) : photomicrograph of heart in rabbits. A-D. Sections belonging to the MSG & ALA treated rabbits group, which shows near the normal histological organized, lesser hemorrhage part (he) and odema (od) of epicardium, myocardium, different type infiltration of inflammatory cells (inf) in three cardial layers, with sparse myocardial fibrosis (fib.) and Stained with H&E, **B, C** stained with MTKR **,A&D** .

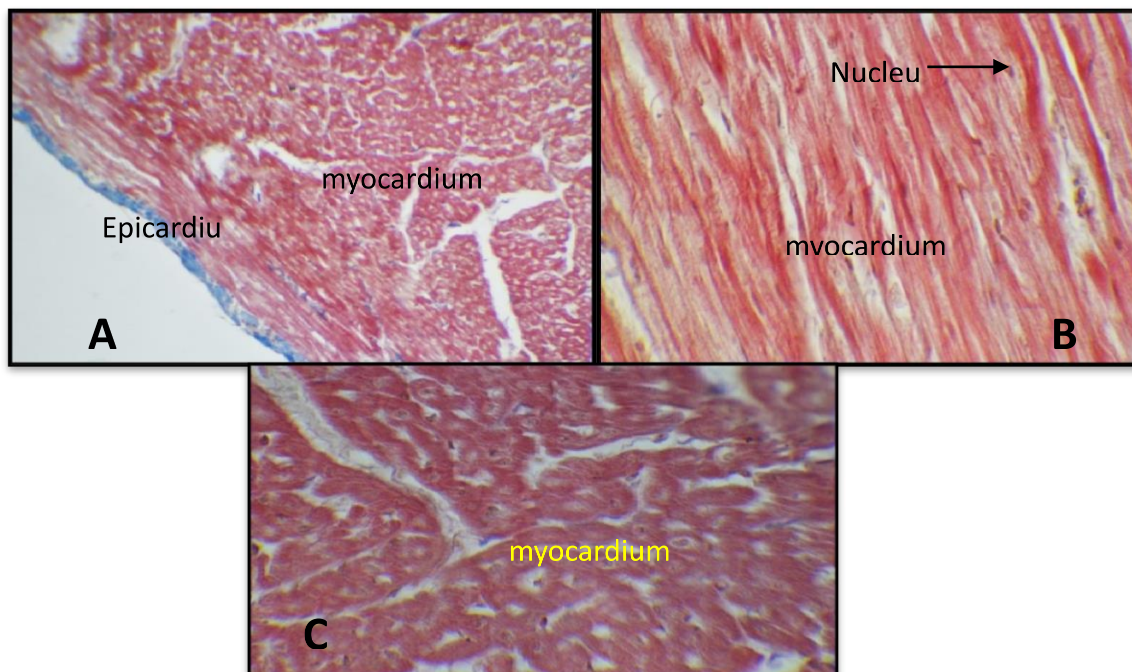


Figure (4.12) : photomicrograph of heart in rabbits: A-C. Sections belonging to the ALA treated rabbits group, that appear assembly control organized. Stained with MTKR **Magnification: A** 10**X, B, and C 40 X**.

CHAPTER FIVE

DISCUSSIONS

5.Discussion

The present study showed that oral intubation of 60mg/kg B.W daily in 10weeks of ALA could offered from cardioprotective against cardiac and aortic damage induced by excess of oral intubation of MSG on male rabbits .

5.1Effect of ALA on the cardiac biomarkers (Troponin I , Endothelin -1 , NO) on the damage induced by MSG in male rabbits .

5.1.1 Cardiac troponin I

The result in table (4.1) showed a significant increase in Cardiac troponin I(cTnI) in GII received MSG as comparing with other groups .The present study is agreed with result conducted by **(Amira *et al* .,2016)** .

Cardiac troponin were proposed as marker of cardiac cell death, both proteins were now widely used and established as the guideline recommended markers in order to assist in the diagnosis of acute myocardial infarction. There were no similar comparable previous studies that reported cTnI level in the serum of MSG treated rabbits. Increased cTnI could be attributed to the nature of cTnI, it is a low molecular weight protein constituent of myofibrillary contractile apparatus of cardiac muscle **(Alpert *et al*., 2000 and Saravanan *et al*., 2013)**. Elevated cTnI levels can predicted risk of myocardial ischemia in the stress subjected patients cardiac cell death and subsequent infarction, this is increased serum cTnI levels in the MSG treated rabbits marked elevations in serum CK-MB activity were registered ,this might be related to concentrations of diagnostic markers of myocardial damage that is released into the extracellular fluid once myocardial cells are damaged **(Priscilla and Prince, 2009; Sabatine *et al*.,2009and Upaganlawar *et al*., 2009).** On exposure to insufficient oxygen supply or nutrient cardiac membrane becomes permeable or may rupture resulting in leakage of

cytosolic enzyme into blood stream with concomitant increase of their serum concentration **(Mathew *et al*., 1985).**

A significant decrease in Cardiac troponin I($P<0.05$) in group GIII received ALA in comparison with other groups . The result of the study agree with result of **(Raffaele Marfella *et al* .,2016)** . ALA reduces oxidative stress and inflammations and leading to the improvement of the adrenergic cardiac innervations , may control complex molecular pathways involved in (Left Ventricular)LV coronary flows distribution in absence of coronary vessel obstruction . For these reasons , ALA orally may be the target treatment to restore a defective LV segments vascularization in treated rabbits .

5.1.2Endothelin-1

The data in table (4.1) showed a significant increase in Endothelin-1($P<0.05$) in GII received MSG in comparison with control group . The results of present study agree with result of **(Kleniewska and Goraca ,2016).**

Endothelin-1 secreted by endothelial cells ,plasma concentration of endothelin-1 ET-1 are increased two to threefold in patient with heart failure irrespective of etiology **(McMurray *et al* .,1992)**. Endothelins are a family of peptides (ET-1, ET-2, and the ET-3) which have different biological activities in both vascular and non-vascular tissues .Endothelin-1 is a 21-amino-acid polypeptide produced primarily by vascular endothelial cells and is characterized as a powerful smooth muscles vasoconstrictor and mitogens **(Galie *et al.,* 2004)**.

ET-1 may lead to oxidative stresses by reducing glutathione, diminishing the antioxidant GSH/GSSG ratio and stimulating lipid peroxidation in a time-dependent manners **(Viswanatha *et al.,* 2011and Murray *et al.,* 2004)**. Increase in vascular permeability and myocardial water content .This finding clearly demonstrated the development of heart injury due to acute inflammations and

interstitial edema in the rabbit heart the same table revealed a significant decrease in Endothelin-1($P<0.05$) in ALA group GIII in comparison with GII. The result of the study agree with **(Hani and Abdesalam ,2019)** Alpha lipoic acid has been also found to be effective in the treatment of various oxidative stress models **(Blumus *et al.,* 2013 and Kawther ,2019)**. Moreover it was a natural substance which was synthesized through enzymatic reactions in plants and animals mitochondria from lipoic acid precursors name lyoctanoic acid and cysteine. It also commonly found in dietary components which inhibits oxidative stress by scavenging free radicals **(Armagan *et al.,* 2015)**. NO degradation in vessel cells and excessive production of endothelin-1 which in turn impairs endothelium – dependent vasodilation **(Vasdev *et al*.,2011)**. Oxidative stress plays a key role in the development of many cardiovascular diseases ,including atherosclerosis , hypertension and heart failure **(Paravicini and Touyz ,2008)**it has been found that ALA prevented eNOS and neuronal NOs but decreased inducible NOs , Meanwhile , it is decreased expression levels of ET-1 **(Bae ,2008).**

5.1.3 Nitric Oxide .

A significant decrease in NO in GII received MSG in comparison with control group .The result of the study is in agreement with result of**(Lobato *et al* .,2010).**

Endogenous nitric oxide(NO)generated in the vascular endothelial cells via endothelial NO-synthase(eNOs) activation and by NO release from NO donor compounds in the modulation of the sGC-cGMP-PKG pathway (soluble guanylyl_cyclase,cyclicGMP,protein kinaseG) and consequent induction of vasodilation .NO production is regulated by eNOS activation in response to

increased cytosolic Ca concentration in the endothelial cell that bind to calmodulin in order to activate eNOS .It has the ability to induce [Ca]increase in endothelial cells with consequent [Ca]decrease in the vascular smooth muscle cells**(Oliveira *et al* .,2009)**

MSG showed enhanced ROS liberation along with decreased NO production because NO-dependent relaxation and NO production were decreased in oxidative stress condition .We measured eNOS protein levels. Although decreased endothelium-dependent relaxation and NO production were found, eNOS protein expression was increased. These results are in agreement with another report showing that in type 2 diabetes, This indicates that the decreased NO- dependent relaxation in MSG rats is not due to decreased expression of eNOS but instead is related to the decreased ability of the enzyme to produce NO **(Villar *et al* .,2006).**

NO is considered the main mediator of endothelium-dependent relaxation . PGI2 and EDHF are also important regulators of vascular activity ,particularly in resistance vessel , although the mechanism of vasodilation induced by the different EDRFs differs , each individual mediators possesses. The capacity to interact with component involve in the synthesis /activation of other mediators and control the activity . there is clear evidence the EDRFs work cooperatively in a complex but integrated manner to maintain homeostasis to the vasculatures**.**

NO may initiate the expression of antioxidant enzymes and nitrosative stress resistance genes . NO enhances the antioxidant potency of GSH by forming S-nitrosoglutathione that is about 100 fold more potent than that of GSH**(Villar *et al* .,2006)**.

The data in table (4.1) showed significant increase ($p<0.05$) in GIII received ALA in comparison with treated groups .the result of the study agreement with result of (**Victoria *et al* .,2018).**

ALA improves the redox states of plasma and endothelium-dependent vasodilation . ALA also induce phosphorylation in human umbilical vascular endothelial cells and the human monocytic leukemia cell line-1 **(Golbidi *et al*.,2011)** . Studies suggest that improved endothelial function due to ALA is at least partially attributed to recoupling of eNOS and increased NO bioavailability**(Golbidi ,2011)**. The antioxidant effect of NO it acts through its effects on vascular smooth muscle, by improving microcirculation and maintaining of organ blood flow and blood pressure regulation . ALA can scavenge a number of free radicals in hydrophilic and lipophilic environments and found to be capable of regenerating many endogenous antioxidants in the body **(El-Sayed *et al* .,2017)**

5.2Effect of ALA on some enzymes (ATP synthase and NO synthase) activity on the damage induced by MSG in male rabbits .

5.2.1 **ATP synthase**

The data in table(4.2) showed a significant increase in ATP synthase ($p<0.05$) in GII group compared with other groups . The result of the study agree with (**Simona *et al*., 2013).** It recently showed that EAATs are also expressed within mitochondria in various tissues (i.e., brain, heart) and cell lines. Such contribute to the Glutamate -stimulated ATP synthesis. Notably, the mitochondrial EAAT dependent Glutamate entry route is regulated by mitochondrial NCX **(Magi *et al*., 2012)**. Glu is an important substance for the average metabolisms in the heart.

Several studies have proposed that glutamate plays an significant role in the convalescing of cardiac oxidative metabolisms after ischemia **(Kugler, 2004and King *et al*., 2001)** and supposed in heart, the noticed metabolic response triggered by Glutamate influx (**Menick *et al*., 2007**). One possibility is that inability to up-regulate the ATP synthase in vivo may lead to an inability to match ATP supply to demand in the heart , and thence to heart failure. ATP levels have been shown to fall significantly in cardiomyocyte. This noticed emphasizes the significant of maintenance the mitochondrial ATP synthase in the regulation of energy balance in the heart cells.

Alpha lipoic acid acid-dependent activation of the oligomycin- sensitive mitochondrial SH-groups in the heart mitochondria and mitoplasts was observed , concomitantly, with an activation of ATP-synthase and a decrease of ATPase activity .Furthermore , the results have been presented suggesting that the oxidation of some vicinal thiols by lipoic acid may underlie the release of Ca2 from the liver mitochondria exposed to lipoic acid reactions leading to the production of cellular energy (ATP) ATP significant decrease ($p<0.05$) in group GIII recessived ALA compare with GII . The result of the study agree with (**Hamzawy ,2014**)

ALA is a disulfide compound that functions as a coenzyme in pyruvate dehydrogenase and alpha–ketoglutarate dehydrogenase mitochondrial. this antioxidant conservation mitochondrial complexes from the NO-induced harmful. The diminished mitochondrial enzyme function central to energy production, due to nitrosative stress . When ALA was introduced , it significantly reduced S-nitrosylation in both of these mitochondrial enzyme complexes and effectively restore mitochondrial enzyme activities inhibited by excess NO, while significantly increasing ATP production.

Alpha-lipoic acid has shown considerable scavenging free radical and ROS-scavenging capabilities along with a proven clinical safety record . The result of the study agreement with result of(**Hamzawy ,2014)** .

5.2.2Nitric oxide synthase

The results in table (4.2) showed a significant decrease in NO synthase ($p<0.05$) in GII received MSG in comparison with treated and controls group this result agreement with **(Lobato *et al*.,2010).**

ROS generation can reduce endothelium-dependent vasodilatation by impairing NO bioavailability .On esource of ROS is uncoupled eNOS. eNOS uncoupling is a processing which eNOS generate superoxide(O2_) when the concentration of either L-arginine the substrate of NOS a cofactor of the enzyme is depleted (**Fo¨rstermann and Munzel ,2006).**

NO –dependent relaxation and NO products measured decreased in MSG decreased eNOS protein rate , although decreased endothelium –dependent relaxation and No production were found eNOs is protein expression was increased .These result of search are in agreement with other reports . The expression of eNOs is paradoxically increased rather than decreased . This indicated that the decreased No –dependent relaxation in MSG is not due to decreased expression of eNOS but instead is related to the decreased ability of the enzyme to produce NO. It Shows significant increase in NO synthase ($p<0.05$) in GIII recessive ALA in comparison with treated and control group. The result of the study agree with result revealed by (**Dong and Kang ,2007**), the expression of the eNOs after ALA supplementation in addition to the direct scavenging activity ALA may modulate endogenous No bioactivity owing to its known ability to increase intracellular level of glutathione .

5. 3 Effect of ALA on some serum oxidant (ONOO , MDA) and antioxidant(GSH) on the damage induced by MSG in male rabbits.

5.3.1 Serum Peroxynitrite (ONOO)

The data in table (4.3) showed a significant increase ($p<0.05$) (ONOO) in GII received MSG in comparison with GI, GIII and GIV and a signifiacnt decrease in GIII received ALA compared with GII the present study agreement with(**PACHER PAL et al.,2007)**

MSG causes oxidative stress which is appears by an important increase in rates of lipid peroxidation . Peroxynitrate itself is also strong oxidant and can react with electron rich groups Peroxynitrate can also be formed through superoxide react NO to formed Peroxynitrate . Increased

glutamate release is sustained activation of glutamate receptors ,and increased accumulation of calcium (Ca+2). There is direct evidence that activation of glutamate receptors and the Ca+2 influx induces the formation of reactive oxygen species, superoxide anion and hydrogen peroxide .Oxidative stress initiates lipid peroxidation cascades that leads to the damage of highly vulnerable cell membranes during the first few days after injury . ONOO has been shown to trigger apoptosis in the cardiomyocytes **(Arstall *et al*., 1999)** , as well as endothelial and the vascular smooth muscle cell, induce decrease in spontaneous contractions of the cardiomyocytes and cause irreversible inhibition of mitochondrial respiratory chain . Peroxynitrite also activates MMPs and nuclear enzyme PARP, which contribute which contributed to impaired cardiovascular functions in most cardiovascular disease and inflammatory disorders **(Jagtap *et al*.,2005).**

Peroxynitrate_activate ERK, a MAPK has been linked with hypertrophic and antiapoptotic response in the heart and inhibit NF_B activation triggered via inflammatory stimuli in cardiac and endothelial cell lines . Furthermore, it induces

the upregulation of adhesion molecule in endothelial cells, the disruption of endothelial glycocalyx and may enhance the adhesion of the neutrophils to endothelium, through complex interactions with various cell signaling pathway and depending on the environment can stimulate or inhibit platelet aggregation. Alpha lipoic acid has free radical scavenger properties and direct antioxidant effects on the recycling of other cellular antioxidants. Alpha lipoic acid has been also found to be effective in the treatment of various oxidative stress models, such as ischemia-reperfusion, diabetes, cataract formation, neurodegeneration, and radiation injury **(Bilska and Wlodek ,2005)** . Therefore, ALA and other antioxidants may reduce harmful effects of oxidative stress**(Emmez *et al* .,2010).**

5.3.2 Malonydialdehyde (MDA)

The data in table (4.3) showed a significant increase ($P<0.05$) MDA in group GII received MSG in compare with GIII group .The result of the current study agree with **(Farombi and Onyeme ,2006 ; Ali *et al* .,2016 and Zeinab *et al* .,2014)**

The present study showed that treated MSG caused an increase in serum MDA , as a result of chronic oral intubation of MSG causes oxidative stress which is manifested by significant increase in levels of lipid peroxidation as evidenced by increased levels of MDA and by the decrease superoxide dismutase and reduced glutathione, glutathione peroxidase and glutathione S-transferase **(Farombi ,2006**). MDA is an evidence of lipid peroxidation promote by iron overload . This study revealed that MSG supplementation generates free radicals and a depletion of anti-oxidants in the thymus and spleen a precursor to the pathogenesis of many diseases **(Pavlovic *et al*.,2007).**

They present study showed the chronic oral intubation of MSG causes oxidative stress which is demonstrated by important increase in the levels of lipid peroxidation as shown by increased levels of MDA in heart (**Singh and Pushpa ,2005)**

MDA significantly decrease ($p<0.05$) in GIII received ALA group in comparison with(GI , GII and GIV)groups.The result of the study is in agreement with result of **(Yasser *et al*.,2015).**

ALA is a hydrophilic and hydrophobic characteristics , being extensively distributed in plants and animals tissues in cellular membranes and in the cytosol**(Deng *et al*.,2013)** .

Metabolic therapy of the ALA is an under-utilised method for the treatment of different heart diseases. This form of therapy differs fundamentally from standard cardiovascular pharmacological therapy. It involves the administration of a substance normally occurring in the body in order to favourably influence metabolic reactions occurring within the cell, by improving cellular energy production and suppressing free radical generation. Lipoic acid as antioxidant efficiently cross the blood brain barrier to accumulate in several brain regions (**Pannerselvam *et al*., 2002)**.It reduces the amount of the hydroxyl radical that was generated by Fenton reaction, and it also scavenges the peroxide and the superoxide radical . ALA can scavenge a number of free radicals in different environments .It is found to be capable of regenerating many endogenous antioxidants in the body **(Bustamante *et al*.,1998)** The protective role of ALA on lipid peroxidation status .It may be attributed to the bioactivity of lipoic acid to directly react with oxidation ,as well as its ability to interfere with the oxidation processes in the lipid and aqueous cellular compartment **(Packer / ., 1997).** On administration of lipoic acid the level of lipid peroxidation in plasma liver and brain was significantly decreased so after administration of lipoic acid the

antioxidant was primarily accumulated in the liver, heart and skeletal muscle after which it efficiently crosses the blood brain barrier to accumulate in several brain regions (**Arivazhagan and Panneerselvam , 2002).**

5.3.3 Glutathione (GSH)

The result showed a significant decrease ($p<0.05$) serum levels of GSH in GII received MSG compared to the control group. This result is in agreement with **(Ali *et al* ., 2016).**

Oral intubation of the MSG 8mg/kg BW daily in 10weeks induced ROS production in the body could be explained by the glutamate induce an increase in the activity of α Ketoglutarate dehydrogenase to potential ROS generator .Moreover , an increased intracellular calcium level via glutamate receptors can stimulate free radical generation and inhibition of cysteine uptake leading to decreased GSH levels that may increase ROS –induced renal cell damaged(Sharma .,2015).Researches have been recorded decreased GSH pool through cytotoxicity and oxidative stress state **(Womgcharoen *et al* .,2012).**

In a study involving isolated rabbits organ exogenous GSH shown to protect organ damage , furthermore, the GSH perfusion medium resulted in an increase in the intracellular level in both damaged and healthy organ by entering the directly or indirectly into the cells **(Ali *et al* ., 2016)**.

Glutathione , an endogen antioxidants reduced in the oxidative status as signalizing of oxidative stresses , reflecting the redox commensuration between oxidation and antioxidations .Various oxidants and antioxidants have been additive effect on oxidative status study appeared that administrated MSG lead

to decreased cardiac reduced glutathione (GSH) level in the serum **(Singh and Pushpa ,2005**).

GSH significant increase ($p<0.05$) in GIII received ALA group in comparison with GII group the result of the study agree with the result of ALA supplementation resulted in increases in total antioxidant capacity GSH and reduced MDA **(Şehirli ,2008and Golbidi *et al* .,2011).** ALA an essential co-factor for mitochondrial energy metabolism, is a natural product that can be synthesized in mitochondria by lipoic acid synthase and also can be absorbed from the diet. It is both hydrophilic and hyrdophobic, and therefore it can easily be absorbed and can easily be distributed into all tissues of body. ALA displays its antioxidant activity especially through GSH activity. **(Sarper *et al*.,2018).**

Other study demonstrated the beneficial effects of ALA in reducing the age-associated alterations in GSH impossible delivery of exogenous GSH to tissues such as the brain and heart. **(Suh *et al*.,2004)** The bioavailability of cysteine delivery agents (e.g., *N*-acetylcysteine) is low. However, ALA can modulate the age-related alteration in GSH levels as it is easily taken up into neural tissues.It was suggested that ALA provides resulted in increase in total antioxidant capacity and reduced GSH. Some in vivo and in vitro experimental trials showed that ALA administration increased the intracellular GSH level by 30–70%**(Kocaoğlu *et al*.,2017).**

5.4 Effect of ALA on Body weight and Heart weight on the damage induced by MSG in male rabbits.

5.4.1Body weight

Significant increase in the body weight GII group in the current study agree with **(Francis *et al* .,2016**) .

MSG is a flavor enhancer largely used in the food industry with individual consumption steadily increasing worldwide. Recent cross sectional and longitudinal studies in healthy Chinese subjects correlated MSG intake with an increased risk of being overweight irrespective of the total calorie intake and physical activity**(He *et al*.,2011)**. Appetite is a complex mixture of physiological and psychological phenomena which include feelings of hunger ,total energy intake, ingestion of particular nutrients, specific cravings and food preferences ,**(Blundell *et al*.,1993)** reported increased food palatability with appropriate concentrations of MSG **(Moore,2003)** reported that during the stimulation of the or sensory receptors, monosodium glutamate influences the appetite positively and induce weight gain in addition to improving the palatability of meals .The increased feed intake and the resultant proportionate increase in the weight gained by the animals could be due to improved appetite enhancement as a result of the positive influence of MSG on the appetite control center of the brain as observed **(Reddy *et al* .,1986 and Hermanussen ., 2006)**suggest a possible link between MSG and overweight /obesity .possible mechanism includes the increasing palatability and food intake ,which sequentially enhance energy balance or caloric storage ,therefore increased body weight .

Another mechanism by disrupting the hypothalamic signaling cascade of leptin action(**Kondoh and Torri , 2008**). That consumption of MSG increases body weight gained explained that the potential for MSG-obesity link lies in the alteration of regulatory mechanism that may affect fat metabolism conducted by(**Halpern ,2000).**

Body weight significantly decreases ($p<0.05$) in GIII received Alpha lipoic acid in comparison with the control group as shown in table (4.4**). (Barky *et al* .,2017 and Carbonelli *et al* .,2011)** . ALA stimulates the reduction of weight, lipid mass and waist circumference in overweight and obese subjects or could be lead to the increased satiety , also α-lipoic acid could induce weight loss by reducing food intake and stimulating energy expenditure with **(Barky *et al* .,2017 and Carbonelli *et al* .,2011) .**

5.4.2 Heart weight

Heart weight significantly elevated ($p<0.05$) in group GII recessived Monosodium glutamate in comparison with the group as shown in table (4.4). The present result agreement with **(Hien *et al*.,2013).**

The increase in weight of the heart of the rabbits treated with 8mg/kg body weight of MSG for10 weeks is suggest to enlargement of the heart. Cardiac enlargement is an increase in the size of the heart rabbit , which involves either hypertrophy or dilation . Hypertrophy involves an increase in the thickness of heart muscles and this usually occurs in one chamber of heart (left ventricle). In most cases cardiac enlargement is abnormal and accompanied by additional cardiovascular problems (**Okon *et al* .,2013**). Hypertrophy of the heart occurs in response to increased stress on the heart the most common causes are related to increased blood pressure in the body and is the most frequent cause of Left Ventricular Hypertrophy (LVH) or Hypertrophy Cardiomyopathy (HCM), other

causes include genetics, infection, diabetes as well as substance abuse in which MSG can be describe as one. The extra work of pumping blood against the increased pressure causes the ventricle to thicken over time, the same way skeletal muscle increases in mass in response to weightlifting **(Kang and Izumo ,2000).**

Heart weight significantly in this result agree with study conducted by **(thirunva and A.T ,2004)** .In the present study. ALA prevents cardiac hypertrophy and oxidative stress in the heart tissue together with a favorable effect on cardiac antioxidant status and lipid profiles .ALA deserves further clinical investigations to investigate its use as a potential supplement in the treatment of CVD .

Alpha-lipoic acid is a cofactor for α-ketoglutarate dehydrogenase and pyruvate dehydrogenase activity. It is required for the oxidative decarboxylation of pyruvate to acetyl coenzyme A, which has links citric acid cycle and glycolysis. Both ALA and dihydrolipoic acid (DHLA) are considered as potent antioxidants with the ability to regenerating other factors such as vitamins C and E in addition to raising glutathione (GSH) intracellularly **(Vallianou *et al*.,2009).**

5. 5.Effect of ALA on the lipid profile on the damage induced by MSG in male rabbits .

5.5.1 lipid profile (VLDL-C,LDL-C, HDL-C,TC and TAG.

The data in table (4.5) showed a significant increase in VLDL-C LDL-C TC significant decrease in HDL in GII group received Monosodium glutamate in compare with other groups. The result of the study agree with study conducted by **(Tonkla *et al*.,2012 and Ford *et al* .,2002)** .

MSG caused reduction in the secretion of growth hormones ,leading to stunted growth and irreversibility in obesity, excessive weight essentially due to the accumulation of fats in adipose tissue arising from high cholesterol level leading

to cardiovascular diseases and correlated with(Body Mass Index) BMI **(He *et al*.,2008)**

MSG may enhance shifting the dietary glucose toward lipid synthesis , increase the rate of the lipogenesis. The activating gene expression of enzymes involve in lipid biosynthesis and storage in adipose tissue **(Collison *et al*.,2009).**

MSG intake is associated with the risk of having metabolic syndrome. The metabolic syndrome is currently considered as the most prominent risk factor for cardiovascular disease world-wide. Data from consider Third National Health and the Nutrition Examination Survey (NHANES III) on 8,814 men and women suggest that 20 to 30% of adults in developed countries meet the syndrome criteria **(Ford *et al*.,2002).**

The results

has shown a significant decrease in LDL , VLDL , Cholesterol , Triglyceride ($p<0.05$) in GIII received Alpha lipoic acid group and significant increase in HDL compare with GI and GII groups .The result of the study agree with result of **(Beata and Anna ,2015)**

Many animals and human studies report that ALA supplementation can result in reduced serum bad lipid profile **(Rideout *et al*.,2015).** prevent serum V LDL –C oxidation by reducing the concentrations of LDL-C, serum TC, and lipoprotein (a) [Lp(a)], as well as other oxidative biomarkers **(Catapano *et al*.,2000).**
Clinical studies confirm that daily oral intubation of ALA can reduce the aortic expression of the adhesion molecules and the accumulation of aortic macrophages and pro inflammatory cytokines, resulting in decreased LDL level and triglyceride concentration and increase HDL, a decrease in lipid peroxidation, serum cholesterol, triglycerides, LDL-c and an increase in HDL-c **(Yang *et al* .,2008)**. Moreover, when ALA was administered ,it prevent the elevated lipid level

(**Arivaxhagan** ***et al*****.,2003**). This action of ALA might be related to the increase rate of lipolysis by increasing of plasma lipase activity at the same periods of decrease plasma triglyceride **(Bennani** ***et al*****.,2000)**. In animal model of ALA daily supplementation caused reduced oxidative stress and improved vascular reactivity in animals fed with a high cholesterol diet may have the ability of initiating LDL receptor synthesized in the liver, resulting in increased return of cholesterol to the hepatic system and elevated synthesis of apoprotein A component for reversed cholesterol transport. ALA has a direct lipid modulating action and an indirect effects on the serum lipid levels **(Harding** ***et al*** **.,2012) .**

Reported decrease in the serum TC, TG, and LDL-C levels in obese subjects, following ALA supplementation . The probable mechanism for these alterations is increasing of insulin sensitivity and controlled activities of the enzymes involved in lipolysis and triglyceride synthesis **(Saltiel and Khan ,2001and Zhang ,2011)** .

5.6 Effect of ALA on the some serum minerals on the damage induced by MSG in male rabbits

5.6.1 Minerals [K, Na and Ca]

The data in table (4.6) showed a significant decrease in serum [K+] concentration in GII received MSG in comparison with another groups(GI,GIII,GIV)and showed a significant increase in [Na+]hypernatremia in GII received MSG in comparing with other groups(GI,GIII,GIV) while there is no-significant in [Ca++] in GII received MSG in comparison with other groups(GI,GIII,GIV).

The three cations potassium, calcium and sodium must be presented in fairly inflexible concentrations if the heart is beats normally; magnesium and the anion

content of extracellular fluid may be important also but they are beyond the scope of this review. As might be expected from their effect on cardiac muscle, potassium and calcium when present in abnormal amounts, produce changes in the electrocardiogram, the former is dramatic and specific, the latter is less ostentatious but none the less characteristic(**Harrigan *et al* .,1999 and Joshi AK *et al*.,2004)**

Hypokalaemia, defined as potassium concentrations of less than 3.5mmol/L, is a common electrolyte disturbance **(Thompson ,2005),** which increases arrhythmias particularly in patients with pre-existing heart disease and those on digoxin. Hypokalaemia greatly increases digoxin's toxicity, and digoxin and hypokalemia in combination reduce the excitability of the myocardial membrane. Even slightly reduced potassium levels can precipitate toxicity resulting in a slow and irregular pulse.

Acute catecholamine release, which occurs in the early stages of myocardial infarction (Ml), can cause sudden potassium shifts and acute hypokalaemia. Therefore with cardiac disease, plasma levels of at least 4,0mmol/L **(Connaughton, 2001),** There is also a clear relationship between hypokalaemia and the frequency of ventricular fibrillation (VF) in acute Ml (Potassium is lost from the body through the kidneys, gastrointestinal tract and skin, but the kidneys are the primary regulators and adjust the amount of potassium excreted in the urine(**Melanie,2007**).

Depletion in [K+]concentration called Hypokalemia , it is the most common encountered clinically as a complication of diuretic therapy2 used to treat hypertension, heart failure, renal disease, and other conditions lower and higher values of serum [K+] have electrophysiological effects that commonly promote cardiacarrhythmias, which effects the cellular balances of K+, Na+, and Ca2+ are interlinked through Na+-K+ ATPase and Na+-Ca2+ exchange **(Howarth , 2005).**

As the major intracellular cation, [K+] is concentrated 30- to 40-fold over its extracellular concentration by the activity of Na+-K+ ATPase in the plasma membrane . In excitable tissues such as the heart, the negative resting Em stabilizes working atrial and Em ventricular myocytes during diastole, preventing spontaneous action potentials (APs) from causing premature extrasystoles.

Depletion in the[K] could be responsible for the increase in the membrane repolarization ,Low extracellular K+ [K+e] leads to hyperpolarization of the resting membrane potential, which paradoxically increases excitability of cardiomyocytes (**Bers, 2001).**

Heart muscle is susceptible in some way to potassium not shared by skeletal muscle, with excessive or increasing concentration of potassium in the serum .

hypo- and hyperkalemia directly impact Na+ and Ca2+ and K+ balances.Voltage-dependent Na1 channels represent a major component of the cycle linking NMDA receptor activity to glutamate release. **(Paul et al.,1996 and Takaoka , 2002)**

This finding provides a tangible explanation for the neuroprotective effects of Na1 channel inhibitors in cerebral ischemia *in vivo* and is consistent with results showing that these agents diminish the associated release of glutamate **(Smith *et al.*, 1993 and Taylor and Meldrum, 1995**).

Intracellular[Na+] is increased in both human and animal models of hypertrophy and HF ,increased Na+ in the subsarcolemmal space in cells with cardiac hypertrophy .It has been theorized that the Na+ channel blockers can cause slowed intraventricular conduction, unidirectional block, the development of a reentrant circuit, and a resulting ventricular tachycardia . These rhythms then can degenerate into ventricular fibrillation. Because many of the Na+ channel blocking agents are also anticholinergic or sympathomimetic agents, bradydysrhythmias are rare.Bradycardia may occur because of slowed depolarization of pacemaker cells

that depend on entry of sodium ions. The Na+ channel blocking agents, however, can affect cardiac pacemaker cells.

The link between Na1 channels and extracellular glutamate accumulation. Several possibilities exist, including release by Na1-dependent action potentials and reversed glutamate uptake after intracellular Na1 loading **(Szatkowski and Attwele , 1994).**

GIII group received 60mg/kg BW daily for 10weeks of ALA caused increases in total antioxidant capacity and reduced Na+ activity compare with GII group received excessive oral intubation of MSG **(Şehirli ,2008).**

Calcium can only fulfill the roles of a metabolic regulator and a second messenger if the cytosolic and intramitochondrial Ca2+ concentrations are tightly regulated agreement with result **(Carafoli ,1987**). One mechanism in this regulation is the precise control of membrane permeability for calcium, which is normally very low but increases in an ordered fashion upon cell activation. The second main mechanism is the extrusion of calcium from the cell at the expense of metabolic energy, either in the form of a Ca2+ -dependent ATPase or a 3Na + ICa2+ exchange mechanism whose energy is derived from the Na + gradient, the latter being created by a Na +, K + - ATPase.the third mechanism ,finally ,,is the binding or sequestration of calcium within the cell by both energy-bind dependent and energy dependent mechanism. increase in [Ca2] that could represent a stimulus able to increase cellular ATP content through activation of the mitochondrial Ca2- dependent dehydrogenases **(Denton, 2009).**

This channel resides in heart and smooth muscle cell membranes. The inhibition of this channel prevents movement of calcium from extracellular sites through the cell membrane to intracellular sites. Decreased intracellular calcium within the

myocardial cells results in slowing of conduction, decreased contractility, and decreased cardiac output.

Various mechanisms were suggested to explain these effects inhibition of the calcium-pump or a transport protein, disturbances in mineral metabolism, inactivation of several enzymes (**Oriquat et al .,2012**) .

5.7 Effect of ALA on the electrocardiograph ECG waves on the damage induced by MSG in male rabbits

5.7.1 Electrocardiograph waves (P, QRS and T)

The study showed that oral administration of MSG to male rabbits for 10 weeks lead to significant increase prolongation in (P,QRS,T) waves and(ST,QT)intervals .These results agree with other study conducted by **(Miguel *et al*.,2019)**

Some studies have recently indicated that dietary monosodium glutamate (MSG) is linked to obesity and the development of many disease ,induce cardiovascular changes, such as increases in blood pressure and arrhythmias, including ventricular fibrillation, which may result in sudden death.

Besides its known effects on metabolism, some studies have suggested that MSG is a compound that could foster the development of some diseases such as obesity and diabetes and cardiovascular disease **(Shannon ,2017).**

Despite this evaluation, a hypothesis has recently been raised linking obesity and heart disease to the consumption of elevated amounts of glutamate **(Collison ,2011).** The exposure of the enteroendocrine cell line to dietary concentrations of MSG reduces glucagon-like peptide-1 (GLP-1) secretion reduces beta cell mass. this alterations increase the risk of developing cardiovascular diseases and neuropathies (**Balkau ,2004).**

Because ECGs provide ample information while consuming relatively little space, an ECG is a time/voltage graph of the electrical activity of the heart. Cardiac muscle activity also generates electrical impulses, and muscle contraction, which creates the pulse, usually follows electrical activity. Although there are many factors that can influence cardiac function, measurement of the time of electrical conduction and the voltage involved usually indicates the function, with different parts of the ECG complex representing different stages of conduction .The normal sinus rhythm complex comprises a P wave, PR interval, Q wave, QRS complex, ST segment, T wave . In this study, the P wave, PR interval, Q wave and QRS complex were evaluated because these parameters can be altered in patients (**Woodrow,2010).** The intake of MSG through food, has been associated to cause atrial fibrillation (AF) .Nevertheless, studies have failed to proof a connection between MSG and those symptoms, including the association of MSG with AF, a common cardiac arrhythmia, in self-reported MSG-sensitive patients **(van ,2017).**

Several subtypes of glutamate receptors (GluRs) are widely and differentially expressed in structures and each had a specific distribution the result of electrocardiograph atrial fibrillation induced by MSG in male rabbits because numerous glutamate receptors have been found in skin epidermis ,hearts electrical conduction ,the heart itself MSG are exitotoxin of cardiac and detectable change in heart rate or rhythm after gived MSG , these receptors may be involved in important cardiac functions (such as contraction, rhythm, coronary circulation) and consequently may be implicated in the pathobiology of cardiac diseases . Therefore, the GluRs in the heart could be targets for the effects of MSG being used as food additive and therefore should be considered for the safety evaluation of this flavor enhancer.

Impulses from the SA node spread out across the atrial muscle and are conducted from one muscle cell to the next. The P wave represents atrial depolarization, and

the PR interval includes the P wave and the period of electrical standstill created by the impulse crossing the AV node **(Woodrow, 2010) .** Thus, diets contain excesses of MSG alter atrial depolarization or the conduction of electrical stimuli from the atria to the ventricle.

Disagreement with study showed Variations in the ionized serum calcium concentration produce the characteristic ECG changes that occur with hypercalcemia and hypocalcemia. These changes are almost entirely limited to the duration of the ST segment with no change in the QRS complexes or T waves. High levels of ionized serum calcium shorten the ST segment on the ECG. Conversely, low levels of ionized serum calcium prolong the ST segment. Variations in the QT interval and the QTc duration are caused by variations in the duration of the ST segment. In marked elevation of the ionized serum calcium, no ST segment is present and the T wave starts immediately at the end of the QRS complex **(Jonathan et al.,2009)** .

The present study demonstrates an elevation of ST-segments in MSG group has reported ECG-changes . This could be due to myocardial necrosis accelerated by MSG ,while treated with alpha ALA -induced ameliorate ST-segment interval , suggestive of their cell membrane-protecting effect .The main clinical cardiomyopathy is a higher incidence of cardiac arrhythmias, including ventricular fibrillation, and a higher occurrence of sudden death because of alterations in ventricular repolarization. These alterations may cause changes in cardiovascular physiology and structure, which can be recorded by ECG **(Feuvray and Lopaschuk , 1997)** .

The QRS complex represents ventricular depolarization, and it is the largest component of the sinus rhythm complexes because a large voltage is required for ventricular depolarization. A specialized conduction pathway composed of the His bundle, bundle branches, hemi-branches and Purkinje fibers ensures that impulses travel quickly from the AV node to the ventricular muscle. The QT interval,

representing the total ventricular depolarization and repolarization time, is measured from the beginning of the QT interval to the end of the T wave and should be less than half the time of the preceding R-R interval. Prolonged QT intervals represent delayed repolarization, which may cause tachydysrhythmias and sudden cardiac death **(Straus , 2006).**

A prolonged QRS complex indicates a prolongation of ventricular depolarization, and a prolonged QT interval indicates prolongation of the events between depolarization and repolarization **(Howarth ,2005)**.

In fact, the direct actions of MSG ingestion may also contribute to these heart rhythm disturbances.

The changes in the QRS complexes and, consequently, in the QT and QTc intervals in the animals fed the diet containing MSG are the consequences of hydro electrolytic alterations . In fact, such electrocardiographic alterations may be explained by the polyuria, which is caused by osmotic diuresis as a result of the increase in glycemia **(Howarth ,2005).** This excessive urinary loss causes a decrease in potassium and other electrolytes, which could be responsible for the increase in the membrane repolarization period represented by a prolonged QT interval. The increased QT interval may also be the result of changes in voltage-dependent potassium ion channels .

Lower and higher values of serum [K+] have electrophysiological effects that commonly promote cardiacarrhythmias, not solely because of direct effects of [K+], but also because the cellular balances of [K+], [Na+] and [Ca2+] are interlinked through [Na+]-[K+] ATPase and[Na+]-[Ca2+] ,Low extracellular [K+] [K+] leads to hyperpolarization of the resting membrane potential, which paradoxically increases excitability of cardiomyocytes. This effect is ascribed to an increased number of available [Na+] channels and the reduced ability of IK1 to generate outward current that protects against membrane depolarization **(Bers,**

2001). Studies have reported[Ca2+]overload in intact, beating hearts perfused with low[K+]. One study found that the [Ca2+]- induced[K+] channel was active in hearts exposed to low [K+](corresponding to clinical hypokalemia) and not in hearts exposed to normal[K+]**(Chan *et al*., 2015).**

In fact, diabetes can alter the magnitude of the potassium channels involved in the repolarization process of the cardiomyocyte membrane **(Shimoni *et al*.,2000)** , data presented in this study demonstrate an elevation of ST-segment in G II group, there is an urgent need for the clinical development of safe and infarction .

The present study was initiated to investigate ALA supplementation could offer cardio protection against MSG induced heart disorder , treatment MSG with ALA combination markedly inhibited MSG induced ST-segment elevated suggestive of their cell membrane –protecting effect in MSG .The release of cellular cardiac enzymes is correlated with changes in plasma membrane integrity or permeability as a response to B-adrenergic stimulation .this might be due to damage upon the sarcolemma by the B agonist rendering it leaky.It has been reported that the oral administration of alpha lipoic acid decrease cardiac troponin induce myocardial infarction **(Nayira *et al* .,2009).**

The result of electrocardiograph atrial fibrillation induced by (MSG) in male rabbits because numerous glutamate receptors have been found in skin epidermis, hearts electrical conduction ,the heart itself ,MSG are exitotoxin of cardiac tissue **(Craig and Burkhart ,2009).**

The results demonstrates that short term sodium modulation acutely altering the prevalence of detectable LVH on ECG ,that introduces new angles into relationship between sodium intake and cardiovascular physiology hypothesizing the basis of this phenomenon precip in worthy of consideration while many factors can contribute perceived LVH on an ECG that showing a fundamental influence on

voltage detection by surface ECG electron involve the product of myocardial cell thickness and ventricular chamber diameter **(Anand ,2009).**

Although changes in the ECG dependent on [Ca] and [K] were clearly visible, limitation regarding the accuracy of the ECG was discovered ,an imbalance of [K] resulted to change of ECG . The influence of the change in atrial electrophysiology could not be evaluate because a ventricular cell model was used ,P wave change and change in the conduction properties of the AV node ,arrhythmias triggering phenomena caused by a changed [K](**Nicolas ,2012).**

ALA has been shown to act by directly scavenging free radicals, increasing the activity of catalase and superoxide dismutase, protecting from lipid peroxidation. in the our study ALA treatment did not show any significant clinical effect in terms of AF recurrence or cardioversion rates**(Celestino ,2017).**

Na+ channel blockers result in widening of the QRS complex (**Harrigan *et al* .,1999**). In some cases, the QRS complexes may take the pattern of recognized bundle branch blocks **(Joshi *et al*.,2004).**

The Na+ channel blocking agents, however, can affect cardiac pacemaker cells. Bradycardia may occur because of slowed depolarization of pacemaker cells that depend on entry of sodium ions. In [Na+] channel blocker poisoning by anticholinergic and sympathomimetic drugs, the combination of a wide QRS complex and bradycardia is an ominous sign and may indicate that the Na+ channel blockade is so profound that tachycardia does not occur, despite clinical muscarinic antagonism or adrenergic agonism **(Kolecki *et al* .,1997).**

The HR of rabbits showed significant decreased in HR($p<0.05$) in GII received MSG in comparison with groups(GI,GIII,GIV). These results corroborate those of

(Howarth , 2005). Reported that the HR declined rapidly after the administration of MSG , reaching a new steady state after 10 week,. A decrease in physical activity may partly underlie this reduction in HR. the reduced bradycardic reflex in MSG-treated rabbits could have been due to a decrease in the vagal effect on the heart, which may have indicated impairments in the vagal reserve used during heart rate responses evoked by baroreceptors. If a reduction in this activity occurs, bradycardia is also reduced. Therefore, the attenuation of the bradycardic response may have resulted from reduced responsiveness of the sinus node to parasympathetic **(Miller *et al*.,1999).**

5.8 Histopathological discussion

The control rabbit groups microscopic structures and thickened were measured in pixels of aortic tunica medial were superposes many authors' who reported during your researches in differ model animals **(Horney *et al*.,2010 and Gasser, 2006)**

The many studies were to evaluate the effect induced by monosodium glutamate (MSG) on systems body include cardiovascular system in a rodent, fowls and rats animals these have shown irregular structure of the laminae fibers, thinning and fragmentation of elastic laminae, proliferation of medial smooth muscle, thickened of the aortic artery greatly in tunica intimal**(Jaiswal *et al*.,2012 ;Karina,2013and Kang *et al.*,2010)**. These are similar under line of examination of vascular section stained of large artery by light microscope reveled, greater of adipocytes penetrated connective tissue of adventitial layer, disarranged and deterioration architectural of elastic fibers in tunica media when compared with control rabbit groups.

The diabetic and hypertension rats are treated with ALA the endothelial cells of intimal layer having squamous property and appear smoother with fewer defect so the smooth muscles don't reactively proliferation and organization of the lamina fibers of tunica media **(Balkis *et al.*,2019and Bekir *et al.*,2014)**. Theses results of author resemble our research. In microscopic section examined of the MSG &ALA supplementation , rabbits group show minimal histologic abnormalities, there by highlighting its role protective countering the cytotoxic injury by MSG so, greater effective of ALA on reducing the free radical that coordination with **(Balachandar,2003)**

On the other hand, we showed three layers of the aorta in ALA treated rabbit groups (intima, media and serosa) had of normal microscopical details structures and decreased thickened in aortic media when compared with treated rabbit groups, that related with ameliorate action of ALA so it has antioxidant, anti-inflammatory, anti-neoplastic and anti-proliferative properties that reported by **(kwiecien et al., 2013 and kapoor, 2013)** .

The damaged of the oxidative cardiac structures in rats, induced by the toxicity effect of MSG appeared in higher activity serum marker enzymes, CPK and AST and microscopical examined myocardial lesion and focal myonecrosis**(Womgcharoen *et al.*,2012 ; Suchalatha and Shyamala,2004 ; Ganesan *et al.*, 2010 and Mnaa,2015)**. So the authors reported in adult Wistar rats about that chronic oral administration of MSG caused oxidative stress and microscopical alteration to the cardiac architecture, as congestion, hemorrhage cloudy, necrosis, swelling and separation tissue**(Paul *et al* 2012 and Sesso *et***

***al*.,2005)** . These coordination outcomes result the cardiac specimen histopathological section of rabbits group that are treated with MSG the destroyed and disarranged heart tissues with cellular infiltration and massive vascular hemorrhaged.

Histopathological finding of the used antioxidants (ALA) mixed with (MSG) in the treated rabbit groups', cardiomyopathy heart showed a near normal morphology of cardiac muscle with the absence of necrosis compared to MSG-induced heart **(Paul *et al*., 2012 and Kingsley*et al*.,2013)** . That proves our truth of the study by observed restricted, necrosis ,fibrosis by attenuates collagen accumulation in the heart and return histological alteration appear protective effect of ALA**(Jung *et al*.,2012) .**

The lipoic acid (ALA) is antioxidant . It has been proved to be protective in several animal model and human**(Nella *et al*.,2018) .** This author reported ensure the scientific facts in our research.The new researchers investigation about the effects the MSG on the cardiovascular system are few but ,reported that by **(Kingsley et al.,2013)** .An increase in heart muscles thickness is noticed in the adult male albino rats that suggest occurs during exposed stress by extra work cardiac pumping blood against increase blood pressure **(Kang and Izumo, 2000).**

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the results and discussion obtained from our study, we can conclude the following:-

1. Oral intubation of 8 mg /kg B.W of MSG for 10 weeks caused significant elevates in the serum cardiac troponin I , Endothelin-1 , ATP synthase ,NO synthase , MDA, ONOO and bad lipid profile with depression in serum GSH ,and HDL.
2. Ameliorate effects of the Oral intubation of 60mg/kg B.W daily of ALA for 10weeks by the significant decrease in the serum cardiac troponin I, Endothelin-I ,ATP synthase ,NO synthase and significant elevation in the GSH,HDL and NO.
3. Ameliorate effects of ALA in the serum [K],[Na]and [Ca] concentration is noticed in the GIII
4. The recovery role of the ALA in the electrocardiograph in GIII and GIV groups showed a significant decrease in the P,QRS and T waves compared with group received excessive supplementation of MSG for 10 weeks
5. Histopathological changes through the formation of marked atherosclerotic and cardiac damage is noticed in GII group with protective role of the ALA in the heart and aorta tissue damage .

6.2 Recommendations

Our study recommended the following:-

1. Further studies about protective role of ALA on the body
2. Studies about PCR real time and the DNA sequencer to detect the genetic mutation that may occur in some ionic channels caused by effects of excessive supplementation of MSG .
3. Immune study about potent effect of ALA in the infected animals
4. Further studies regarding other cardiac biomarkers which are not mentioned in this study are recommended
5. Further studies about Cardioprotective role of ALA in the arrhythmias
6. Electron microscopy study of different organs subjected to MSG overload.

REFERENCES

References

Abass MA and Abd El-Haleem MR., (2011): Evaluation of monosodium glutamate induced neurotoxicity and nephrotoxicity in adult male albino rats. Journal of American Science. 7: 264-276.

Adams , J.,(1994) : Diagnosis of perioperative myocardial infarction with of cardiac troponin I .N. Eng .J .Med .330: 670-4.

Al- Zamely OM, Al-Nimer MS, Muslish RK.,(2001): Detection the level of peroxynitrite and related with antioxidant status in the serum of patient with acute myocardial infarction". Nat J Chem . 4: 625-637.

Al-Ali.,(2018): Submitted To The Counical Of The College Of Veterinary Medicine /Role Of Alpha Lipoic Acid On Pituitary –Testicular Function And Gene Expression Of Glutathione Reductase In Hydrogen Peroxide Treated Adult Rats.

Alan, H. B., (2006): Wu: Tietz Clinical Guide to Laboratory Tests, 4th ed. WB Saunders.564.

Ali A. Ahamed, Mohamed M.A.Shahat, Haredy H. Haredy, Hoda k. Abd El-Hady .,(2016): Evaluate the protective effects of some antioxidant agent (β-carotene and Curcumin) against Monosodium Glutamate-induced cardiopathy in male albino rats. International Journal of Advanced Research4:82-92.

Alpert JS, Thygesen K, Antman E., (2000): Myocardial infarction redefined-a consensusdocument of the Joint European Society of Cardiology/ American College of Cardiology Committee for the redefinition of myocardial infarction. Journal of the American College of Cardiology. 36: 959-969.

Amin SB, Ahlfors C.,(2008): Eff ect of storage and freezing on unbound bilirubin measurement. Clin Chim Acta .396:56-7.

Amira A, Wahdan1 and Mohamed M. Shareef.,(2016): Study of the Protective Effect of Vitamin C on Monosodium Glutamate Induced Cardiotoxicity in Adult Male Albino Rats. Ain Shams Journal of Forensic Medicine and Clinical Toxicology. 27: 49- 56.

Anand vaidya .,(2009): Dietary sodium alter the prevalence of electrocardiogram determined left ventricular hypertrophy in hyper tension.AMJ.Hypertens.22(6).669-673.

Arivaszhagan P ,C, Panneerselvam.,(2002): Effect of □ lipoic acid on neural antioxidant in aged rats .Pharm.Res.42:219-22.

Arivaxhagan P, Panneerselvam SR, Panneerselvam C.,(2003) :Effect of DL-alpha-lipoic acid on the status of lipid peroxidation and lipids in aged rats. Journal Gerontol Biological Science Medicine.58(9):788-791.

Arivazhagan, P., & Panneerselvam, C., (2002): Effect of DL-α-Lipoic Acid on the Status of Lipids and Membrane-Bound ATPases in Various Brain Regions of Aged Rats. J. Anti-Aging Med. P: 5(4), 335-343.

Arivazhagan, P., Thangaswamy, T., Ramanathan, K., Kumaran, S., & Pannerselvam, C., (2002): Effect of Dl-a-lipoic acid on the status of lipid peroxidation and protein oxidation in various brain regions of aged rats. Journal of Nutritional Biochemistry.13:619-624.

Armagan, I., Bayram, D., Candan, I. A., Yigit, A., Celik, E., Armagan, H. H., & Uğuz, A. C., (2015): Effects of pentoxifylline and alpha lipoic acid on methotrexate-induced damage in liver and kidney of rats. J. Env. Tox. & phar. P: 39(3), 1122-1131.

Arruda ,N.J.,FILHo,J .,(2003): Simple and in expensive flow L-glutamate determination using pumpkin tissue . J. Agricul. And Food Chemistry .51: 6945-6948.

Arstall MA, Sawyer DB, Fukazawa R, Kelly RA.,(1999): Cytokinemediated apoptosis in cardiac myocytes: the role of inducible NO synthase induction and peroxynitrite generation. *Circ Res* 85:829–840.

Aziz M,Yadav KS.,(2016): Pathogenesis of Atherosclerosis. Med Clin Rev.2:3.

Babuin L, Jaffe AS.,(2005): Troponin: the biomarker of choice for the detection of cardiac injury. Can Med Assoc J.173(10):1191– 1202.

Bae, E.H.,(2008) :Effects of α-lipoic acid on ischemia-reperfusion-induced renal dysfunction in rats. American journal of physiology-renal physiology. 294(1): p. F272-F280.

Balachandar, A. V., Malarkodi, K. P., & Varalakshmi, P.,(2003): Protective role of DLa-lipoic acid against adriamycin-induced cardiac lipid peroxidation. *J. Hum. & exp. Tox. P: 22*(5). 249-25.

Balkau, B.,(2004): Prediction of the Risk of Cardiovascular Mortality Using a Score That Includes Glucose as a Risk Factor. The DECODE Study. Diabetologia , 47, 2118-2128.

Balkis. B., Faizah . O., Louis. S.R., Abu Baker. M ., Radzi. M., Osman. K., (2019): Effect of Alpha Lipoic Acid on Oxidation Stress and Vascular Wall of Diabetic Rats. Romanian Journal of Morphology and Embryology , 50(I): 23-30.

Balligand JL, Ungureanu Longrois D, Simmons WW, Pimental D, Malinski TA, Kapturczak M, Taha Z, Lowenstein CJ, Davidoff AJ, Kelly RA.,(1994):Cytokine-inducible nitric oxide synthase (iNOS) expression in cardiac myocytes. Characterization and regulation of iNOS expression and detection of iNOS activity in single cardiac myocytes in vitro. J Biol Chem.269:27580–27588.

Barky AR, Hussein SA and Mohamed TM.,(2017): The Potent Antioxidant Alpha Lipoic Acid. J Plant Chem and Ecophysiol. 2(1): 1016.

Beart PM, O'Shea RE.,(2007): Transporters for L-izlutamate: an update on their molecular pharmacology and pathological involvement. Br J Pharmacol .150:5-17.

Beata Skibska, Anna Goraca.,(2015) : The Protective Effect of Lipoic Acid on Selected Cardiovascular Diseases Caused by Age-Related Oxidative Stress. Oxidative Medicine and Cellular Longevity. 1: 90-151.

Bekir.U. E., Serap .C .M ., Osman .Y., Pinarak . K.,(2014):The Effect of A-Lipoic Acid on Aorta Injury and Hypertension in The Aorta Remenant Kidney . Faculty Of Medicine ,Dokuz Eylul University

Bennani-Kabchi N, Kehel L, El- Bouayadi F, Fdhil H, Amarti A, Saidi A, Marquie G.,(2000): New model of atherosclerosis in insulin resistant in rats: hypercholesterolemia combined with D2 vitamin, Atherosclerosis. 2000; 150(1):55-61.

Bers, D.M. (2001). Excitation-Contraction Coupling and Cardiac Contractile Force, 2nd Edn. Dordrecht; Boston, MA: Kluwer Academic Publishers.

Beyreuther K, Biesalski HK, Fernstrom JD., (2007): Consensus meeting: Monosodium glutamate - an update. Eur J Clin Nutr. 61: 304-313.

Biewenga GP, G.R ., Haenen A., Bast.,(1997):The pharmacology of the antioxidant lipoic acid .Gen .Pharmacol.29:315-331.

Bilska, A., & Wlodek, L., (2005) :Lipoic acid-the drug of the future. *J. Phar. Rep. P: 57*(5), 570-577.

Blaylock R.,(2007): Food additives : What You Eat Can Kill you .The Blaylock Wellness Report 4.

Blumuş, F. G., Gürsu, M. F., Muz, M. H., Yaman, İ., Bulmuş, Ö., & Sakin, F. (2013): Protective effects of alpha-lipoic acid on oleic acid-induced acute lung injury in rats. J. Bal. med. P: 30(3), 309.

Blundell JE, Lawton CL, Hill AJ.,(1993): Mechanisms of appetite control and their abnormalities in obese patients. Horm Res Paediatr. 39(Suppl 3): 72-76.

Bo K. Siesj and Finn Bengtsson.,(1989): Calcium Fluxes, Calcium Antagonists, and Calcium-Related Pathology in Brain Ischemia, Hypoglycemia, and Spreading Depression. Journal o/ Cerebral Blood Flow an d Metabolism 9:127-140.

Bojanic V, Bojanic Z, Najman S ., (2009): Diltiazem prevention of toxic effects of monosodium glutamate on ovaries in rats. Gen Physiol Biophys. 28: 149-154.

Boon NA, Colledge NR ,Walker BR , Ralston SH ,Ralston Sh.,(2010): Davidsons principles and practice of medicine , 21 edition , CHurchil living stone company.

Bradely G,Kleing .,(2003): Cunninghams Textbook of veterinary physiology.Elsevier.188 198.

Bray MV, WE. C. Weir EC, D. G. Brownstein, M. L. Delano., (1992): Endometrial venous aneurysms in three New Zealand white rabbits. Lab Anim Sci.; 42(4):360-2.

Breddt,D.S. and Snyder ,S.H.,(1994):Annu.Rev .Biochem .63:175-195.

Bustamante J, J.K., Lodge, L., Marcocci, H.J., Tritschler, L., Packer, B.H., Rihn, 1998: α-Lipoic acid in liver metabolism and disease. Free Rad. Biol. Med. 24, 1023–1039.

Caires A, G.S. Fernandes, A.M. Leme, B. Castino, E.A. Pessoa , S.M. Fernandes,C.D. Fonseca, M.F. Vattimo, N. Schor and F.T. Borges.,(2018): Endothelin-1 receptor antagonists protect the kidney against the nephrotoxicity induced by cyclosporine-A in normotensive and hypertensive rats.10:1414-1590.

Carafoli E., (1 987): Intracellular calcium homeostasis. Annu Rev Biochem 5 6 : 3 95-433.

Carbonelli MG, Renzo LD, Bigioni M, Di Daniele N, De Lorenzo A, Fusco MA,(2011):Alpha lipoic acid supplementation: a tool for obesity therapy. Curr Pharm Des. 16: 840-846.

Catapano AL, F. M. Maggi, and E. Tragni.,(2000):Low density lipoprotein oxidation, antioxidants, and atherosclerosis," *CurrentOpinion in Cardiology*, vol. 15, no. 5, pp. 355–363.

Celestino Sardu, MD, MSc.,(2017): Effects of α-Lipoic Acid on Multiple Cytokines and Biomarkers and Recurrence of Atrial Fibrillation within One Year of Catheter Ablation. Am J Cardiol. 119(9): 1382–1386.

Chan, Y. H., Tsai, W. C., Ko, J. S., Yin, D., Chang, P. C., Rubart, M., . Chen, P. S., (2015): Small-conductance calcium-activated potassium current is activated during hypokalemia and masks short-term cardiac memory induced by ventricular pacing. Circulation 132, 1377–1386.

Chang C-L , Liao Jc ,Kuo L.,(1998): Arginase Modulates Nitric Oxide Production In Activated Macrophages .Am J Physiol .274: 342-348.

Chidambaram Prahalathan, Elangovan Selvakumar, Palaninathan Varalakshmi.,(2005): Protective effect of lipoic acid on adriamycin-induced testicular toxicity. Clinica Chimica Acta .360 : 160–166.

Collison KS, Maqbool Z, Saleh SM, Inglis A, Makhoul NJ, Bakheet R, Al-Johi M, Al-Rabiah R, Zaidi MZ, Al-Mohanna FA(2009): Effect of dietary monosodium glutamate on trans fat-induced nonalcoholic fatty liver disease. J Lipid Res . 50:1521–1537.

Collison, K.S., (2011): Sex-Dimorphism in Cardiac Nutrigenomics: Effect of Trans Fat and/or Monosodium Glutamate Consumption. BMC Genomics , 12, 555.

Connaughton M., (2001):Evidence-Based Coronary Care Churchill livingstone, London.

Corp, I., (2011): IBM SPSS statistics for windows, version 22.0. IBM Corp Armonk, NY.

Craig G, Burkhart.,(2009): ‘Lone’ atrial fibrillation precipitated by monosodium glutamate and aspartame. University of Toledo College of Medicine.

David A, HARRIS and Anibh M. DAS.,(1991): Control of mitochondrial ATP synthesis in the heart. Biochem. J. 280:561-573.

David Alonso, Marek W. Radomski.,(2003): The Nitric Oxide-Endothelin-1 Connection. Heart Failure Reviews.8:107–115.

Deng, C., Sun, Z., Tong, G., Yi, W., Ma, L., Zhao, B., Cheng, L., Zhang, J., Cao, F. & Yi, D. (2013). α-Lipoic acid reduces infarct size and preserves cardiac function in rat myocardial ischemia/reperfusion injury through activation of PI3K/Akt/Nrf2 pathway. J. PloS one, P: 8(3), e58371.

Denton RM., (2009): Regulation of mitochondrial dehydrogenases by calcium ions. Biochim Biophys Acta 1787:1309–1316.

Denton, R. M. & McCormack, J. G., (1990) :Annu. Rev. Physiol. 52, 451-466.

Dijk SN, Francis PT, Stratmann GC, Bowen DM., (1995): NMDA-induced glutamate and aspartate release from rat cortical pyramidal neurones: evidence for modulation by a 5-HT1A antagonist. Br J Pharmacol 115:1169 –1174.

Dong Il Kang .,(2007): Effect of Alpha lipoic acid on Nitric oxide synthase expression and ultrastructural changes in the Bladder of Rats with streptozotocin –induced Diabetes .inje university of medicine .

Douglas G Burrin and Barbara Stoll.,(2009): Metabolic fate and function of dietary glutamate in the gut. American Society for Nutrition .(90)850S-6S. **Walker R,** Lupien JR.,(2000): The safety evaluation of monosodium glutamate. In: International Symposium on Glutamate. 130:1049S-1052S.

El-Sayed, E. S. M., Mansour, A. M., & El-Sawy, W. S. (2017). Alpha lipoic acid prevents doxorubicin-induced nephrotoxicity by mitigation of oxidative stress, inflammation, and apoptosis in rats. J. Bio. & Mol. Tox., P: 31(9), e21940.

Emmez H, Yildirim Z, Kale A, Tonge M, Durdag E, Borcek AO, Ucankus LN, Dogulu F, Kilic N, Baykaner MK.,(2010): Anti-apoptotic and neuroprotective effects of alpha-lipoic acid on spinal cord ischemia–reperfusion injury in rabbits. Acta Neurochir 152: 1591–1601.

Esther van Praag.,(2015):Congestive heart failure in rabbits.MediRabbit.

Faizah Bhatti, Richard W. Mankhey, Laureano Asico, Mark T. Quinn, William J. Welch, And Christine Maric .,(2005) : Mechanisms Of Antioxidant And Pro-Oxidant Effects Of A-Lipoic Acid In The Diabetic And Nondiabetic Kidney. Department Of Medicine, Division Of Nephrology And Hypertension. 67 :1371–1380.

Farmobi E, and Onyema O., **(2006):**Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: Modulatory role of vitamin C, vitamin E and quercetin. Hum. Exp. Toxicol., 25: 251-259. 43.

Fasce, C. F., (1982): Serum Cholesterol determined colorimetrically with enzyme. Clin. Chem. 18: 901.

fauci AS,Braounwald E,Dennis L,Stephen L.,(2010): Harison internal medicine ,17th edition by McGraw-Hill Company.

Feuvray, D. and Lopaschuk, G.D., (1997): Controversies on the Sensitivity of the Diabetic Heart to Ischemic Injury: The Sensitivity of the Diabetic Heart to Ischemic Injury Is Decreased. Cardiovascular Research , 34, 113-120.

Filatov VL, Katrukha AG, Bulargina TV.,(1999): Troponin: structure, properties, and mechanism of functioning. Biochemistry (Moscow). 64:1155–1174.

Filer LJ, Stegink LD.,(1994):Report of the proceedings of the glutamate workshop. Critical Review in Food Science and Nutrition. 34(2):159-174.

Fo¨rstermannU,Mu¨nzel T.,(2006):.Endothelialnitricoxidesynthasein vascular disease.Circulation2006;113:1708e14.

Ford ES, Giles WH, Dietz WH.,(2002) Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA .287:356–359.

Fossati, P. and Prencipe, L. ,(1982): Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. 28:2077-2080.

Francis A. Gbore, Olubu R. Olumomi, Irewole M. Aworetan, Ruth A.O. Gabriel-Ajobiewe .,(2016). Oral administration of monosodium glutamate altersgrowth and blood parameters in female rabbits .European Journal of Biological Research . 6 (3): 218-225.

Friedewald ,W.F.,(1972) Clin Chem, 18: 499 – 502.

Fuchus J,H.,Schofer ,R.,Milbradt, H.J ., Freisleben ,R.,Buhul,W.,Siems,T.,Grune.,(1993):Studies on lipoate effects on b;ood redox state in human immunodeficiency virus infected patients Arzneimit Forsche .43:1359-1362.

Galie N, Manes A, Branzi A.,(2004): The Endothelin System In Pulmonary Arterial Hypertension. Cardiovasc Res 61:227-237.

Gasser TC., Ogden RW., And G. A. Holzapfel.,(2006) : Hyperelastic Modelling of Arterial Layers with Distributed Collagen Fiber Orientations. *J. R. Soc. Interface*, Vol, 3: 15–35, Feb.

Ganesan B., Buddhan R., Anandan R., Sivakumar W. and Anbinezhilan R. (2010): Antioxidant defense of betaine against isoprenaline-induced myocardial infarction in rats. Mol. Biol. Rep. 37(3):1319-1327.

Geha RS, Beiser A, Ren C., (2000): Review of alleged reaction to monosodium glutamate and outcome of a multicenter double-blind placebo controlled study. J Nutr. 130: 1058s-1062s.

Ghibu S, Richard C, Vergely C, Zeller M, Cottin Y, Rochette L.,(2009): Antioxidant properties of an endogenous thiol: alpha-lipoic acid, useful in the prevention of cardiovascular disease. J Cardiovasc Pharmacol.54: 391-398.

Golbidi S, Badran M, Laher I.,(2011): Diabetes and alpha lipoic acid. *Front Pharmacol*.2:69.

Goldie, R.G ., (2000) :J. Cardiovasc. Pharmacol. **36**:S228.

Goldmann BU, Christenson RH, Hamm CW, et al.,(2001):Implications of troponin testing in clinical medicine. Curr Control Trials Cardiovasc Med .2:75–84.

Gomes, AV et al., (2002) : The role of Troponin in muscle contraction. Life. 54: 323-333.

Gora¸A,ca, H. Huk-Kolega, A. Piechota, P. Kleniewska, E. Ciejka, and B. Skibska.,(2011): Lipoic acid—biological activity and therapeutic potential," *Pharmacological Reports*.63,:849–858.

Griendling KK and FitzGerald GA (2003): Oxidative stress and cardiovascular injury: Part II: Animal and human studies. Circulation. 108: 2034- 2040.

Grove, T. H., (1979): Effect of reagent pH on determination of high density lipoprotein cholesterol by precipitation with sodium phosphotungstate- magnesium. Clin. Chem. 25: 560-564.

Guyton ,and Hall E.,(2006): Text book of medical physiology ,eleventh dition ,Saunders and Elsevier company , international edition .

Hadj A,S. Pepe ,F. Rosenfefeldt .,(2007): The clinical application of metabolic therapy for cardiovascular disease .Heart ,lung and circulation .S57(16):S56-S64.

Halpern BP.,(2000): Glutamate and the flavour of food. JNutr. 130(4S Suppl): 910S-914S.

Hamzawy, M.A.,(2014): *Hepatoprotective effect of estradiol and-Lipoic Acid in Rats.* Global Journal of Pharmacology. **8**(4): p. 694-702.

Hani M, Abdelsalam.,(2019): Mitigative effects of Alpha-lipoic acid for the toxicity of Dimethoate in male rats. Zoology department- Faculty of science- Zagazig University.

Harding SV, T. C. Rideout, and P. J. H. Jones, (2012):Evidence for using alpha-lipoic acid in reducing lipoprotein and inflammatory related atherosclerotic risk," *Journal of Dietary Supplements*, vol. 9, no. 2: 116–127.

Harrigan RA, Brady WJ.,(1999) :ECG abnormalities in tricyclic antidepressant ingestion. Am J Emerg Med .17(4):387–93.

Haynes WG, Strachan FE, Webb DJ.,(1995): Endothelin ETA and ETB receptors cause vasoconstriction of human resistance and capacitance vessels *in vivo*. *Circulation.*92:357–363.

Haynes WG, Webb DJ.,(1998): Endothelin as a regulator of cardiovascular function in health and disease. J Hypertens.16:1081–1098.

He K, Du S, Xun P, Sharma S, Wang H, Zhai F, Popkin B.,(2011): Consumption of monosodium glutamate in relation to incidence of overweight in Chinese adults: China Health and Nutrition Survey (CHNS). Am J Clin Nutr.93:1328–1336.

He K, Zhao L, Daviglus ML, Dyer AR, Van Horn L, Garside D, Zhu L, Guo D, Wu Y, Zhou B, Stamler J.,(2008): Association of Monosodium Glutamate Intake with overweight in Chinese adults: the INTERMAP study. Obesity . 16:1875–1880.

Henriksen E J, S., Jacob, R.S. Streeper ,, D.L.,Fogt , J.Y., Hokama ,H.j., Tritschler .,(1997): stimulation by -lipoic acid of glucose transport activity in skeletal muscle of lean and obese Zucker rats .Life Sci .61:805-812.

Henry R.F , Harper and Row ,Hargersein, M. D .,(1974): Clinical Chemistry Principle and Technics.2nd Ed .

Hermanussen, M., (2006) :Obesity, Voracity, and Short Stature: The Impact of Glutamate on the Regulation of Appetite. European Journal of Clinical Nutrition,.60,:25-31.

Hien Vuthithu, Andrea Wakita, Saiko Shikanai, Tamami Iwamoto.,(2013). Epidemiological Studies of Monosodium Glutamate and Health. Journal of Nutrition & Food Sciences.ISSN: 2155-9600.

Hoeflemayr ,J.,(1979) : Praxis and Helferin 8.

Horney. L., Kronek. H., Zitny. R ., Vesely . J ., Hilan. M .,(2010): Orientation of Collagen Fiber in Aortic Histological Section .Bulletin Applied Mechanics ,6(22): 25-24.

Howarth, F.C. ,(2005): Long-Term Effects of Streptozotocin-Induced Diabetes on the Electrocardiogram, Physical Activity and Body Temperature in Rats. Experimental Physiology. 90:827-835.

Howarth, F.C., (2005) :Short-Term Effects of Streptozotocin-Induced Diabetes on the Electrocardiogram.Physical Activity and Body Temperature in Rats. Experimental Physiology. 90:237-245.

Iamsaard S, Sukhorum W, Samrid R., (2014): The sensitivity of male rat reproductive organs to monosodium glutamate. Acta Med Acad. 43: 3- 9.

Inoue A, Yanagisawa M, Takuwa Y,Mitsui Y,Kobayashi M, Masaki T.,(1989): The human preproendothelin-1 gene. Complete nucleotide sequence and regulation of expression. J Biol Chem.264:14954–14959.

Iwanaga T, Goto M.,(2005) :Watanabe M. Cellular distribution of glutamate transporters in the gastrointestinal tract of mice: an immunohistochemical and in situ hybridization approach. Biomed Res.26:271-8.

Jaffe AS, Landt Y, Parvin CA.,(1996): Comparative sensitivity of cardiac troponin I and lactate dehydrogenase isoenzymes for diagnosing acute myocardial infarction. Clin Chem .42:1770–1776.

Jagtap PG, Baloglu E, Southan GJ, Mabley JG, Li H, Zhou J, van Duzer J, Salzman AL, Szabo C.,(2005). Discovery of potent poly- (ADP-ribose) polymerase-1 inhibitors from the modification of indeno[1,2-c]isoquinolinone. *J Med Chem* 48: 5100–5103.

Jaiswal. S., Singh. I., Mahant . D., Sathapathy. S., Mrigesh. M., Tamil Selvan. S .,(2012): Histological ,Histomorphometrical ,Histochemical and Ultrastructural on The Heart Uttara Fowl .Journal of Entomology and Zoology Studies , 5(6): 2365-2370.

Janeczko M, Stoll B, Chang X, Guan X, Burrin DG(2007). Extensive gut metabolism limits the intestinal absorption of excessive supplemental dietary glutamate loads in infant pigs. J Nutr.137:2384-2390.

Jassim Al-Hijji, Bengt Larsson,4 and Satish Batra.,(2000): Nitric Oxide Synthase in the Rabbit Uterus and Vagina: Hormonal Regulation and Functional Significance. Biology Of Reproduction .62: 1387–1392 .

Jeqular E .,(2002):leptin signaling ,adiposity,and energy balance ,Ann,N.YAcad .Sci. 967:379-388.

Jonathan D. Kibble, Colby R. Halsey.,(2009): Medical physiology . The McGraw-Hill Companies.153 158.

Joshi AK, Sljapic T, Borghei H.,(2004) :Case of polymorphic ventricular tachycardia in diphenhydramine poisoning. J Cardiovasc Electrophysiol 2004;15(5):591–3.

Juliet AWolfe Barry BSc FRCA,Julian H Barth MD FRCP FRCPath,Simon J Howell FRCA MD.,(2008): Cardiac troponins: their use and relevance in anaesthesia and critical care medicine. Continuing Education in Anaesthesia, Critical Care & Pain .

Jung Eun Lee, Chin-ok Yi, Byeong Tak Jeon, Hyun Joo Shin, Soo Kyoung Kim , Tae Sik Jung,Jun Young Choi and Gu Seob Roh.,(2012): Alpha-lipoic acid attenuates cardiac fibrosis in Otsuka Long-Evans Tokushima Fatty rats. Cardiovascular Diabetology . 11:111.

Kang J.H., Tsuyoshi. G., Han I.S., (2010) :Dietary Capsaicin Reduces Obesity-Induced Insulin Resistance and Hepatic Steatosis in Obese Mice Fed A High-Fat Diet. Obesity (Silver Spring) ,18: 780–787.

Kang PM and Izumo S (2000): Apoptosis and heart failure: A critical review of the literature. Circ Res. 86: 1107-1113.

Kapoor, S., (2013): The anti-neoplastic effects of alpha-lipoic acid: clinical benefits in system tumors besides lung carcinomas. *J. Korean Thor. & Cardi. Sur.*, *P: 46*(2): 162.

Karina.R.E., Beatriz. D., Luis . M.,(2013): Capasicin –Induced Metabolic and Cardiovascular Autonomic Improvement in an Animal Model of The Metabolic Syndrome . Article in The British Journal of Nutrition.

Kaushalya Wijayasekara, Jagath Wansapala.,(2017): Uses, effects and properties of monosodium glutamate (MSG) on food & nutrition. International Journal of Food Science and Nutrition.Volume 2; Issue 3; Page No. 132-143.

Kawther ,Y.J.,(2019):protective effect of α-lipoic acid on reproductive efficacy of male rats treated with gentamycin.

King N, Williams H, McGivan JD, and Suleiman MS., (2001): Characteristics of L-aspartate transport and expression of EAAC-1 in sarcolemmal vesicles and isolated cells from rat heart. Cardiovasc Res 52:84–94.

Kingsley OA, Jacks TW, Amaza DS., (2013): The effect of monosodium glutamate (MSG) on the gross weight of the heart of albino rats. Sch J App Med Sci. 1: 44-47.

Kleniewska P, A. Gorąca.,(2016): Influence Of Endothelin 1 Receptor Blockers And A Nitric Oxide Synthase Inhibitor On Reactive Oxygen Species Formation In Rat Lungs. *Physiol. Res. 65: 789-798.*

Kocaoğlu S, Aktaş Ö, Zengi O.,(2017) :Effects of alpha lipoic acid on motor function and antioxidant enzyme activity of nerve tissue after sciatic nerve crush injury in rats. *Turk Neurosurg.*1:1–8.

Kojda G, Kottenberg K.,(1999): Regulation of basal myocardial function by NO. Cardiovasc Res.41:514–523.

Kolecki PF, Curry SC.,(1997): Poisoning by sodium channel blocking agents. Crit Care Clin . 13(4):829–48.

Kondoh, T. and Torii, K., (2008): MSG Intake Suppresses Weight Gain, Fat Deposition,and Plasma Leptin Levels in Male Sprague-Dawley Rats. Physiology & Behavior .95,:135-144.

Kondoh, T., Mallick, H.N. and Torii, K.,(2009) :Activation of the Gut-Brain Axis by Dietary Glutamate and Physiologic Significance in Energy Homeostasis. The American Journal of Clinical Nutrition, 90, 832S-837S.

Konosu S, Hayashi T.,(1987):Yamaguchi K. Role of extractive components of boiled crab in producing the characteristic flavor. In L. J. Filer, S. Garattini, M. R. Kare, W. A.

Kugler P., (2004) :Expression of glutamate transporters in rat cardiomyocytes and their localization in the T-tubular system. J Histochem Cytochem 52:1385–1392.

Kurihara K.,(2009). Glutamate. From discovery as a food flavor to role as a basic taste (umami). American Journal of Clinical Nutrition. 90:719S-722S.

Kwiecień, B., Dudek, M., Bilska-Wilkosz, A., Knutelska, J., Bednarski, M., Kwiecień, I., Zygmunt, M., Iciek, M., Sokołowska-Jeżewicz, M., Sapa, J. & Włodek, L., (2013):In vivo anti inflammatory activity of lipoic acid derivatives in mice. *J. Post. Hig. Med. Dosw*, *P: 67.* 331-338.

Lobato NS, F.P.Filgueira a, E.H.Akamine, A.P.C.Davel ., (2010) : Obesity induced by neonatal treatment with monosodium glutamate impairs microvascular reactivity in adult rats: Role of NO and prostanoids. journal homepage.

Lostcher, H.R.,(1984): Biochemistry.18:4134.

Luc Rochette, Steliana Ghibu, Adriana Muresan, and Catherine Vergely.,(2015): Alpha-lipoic acid: molecular mechanisms and therapeutic potential in diabetes. Canadian Journal of Physiology and Pharmacology. 1: 1021-1022.

Madej M, Lundh T.,(2002): Lindberg JE. Activity of enzymes involved in energy production in the small intestine during suckling-weaning transition of pigs. Biol Neonate 82:53-60.

Magi S, Lariccia V, Castaldo P, Arcangeli S, Nasti AA, Giordano A, and Amoroso S (2012):Physical and functional interaction of NCX1 and EAAC1 transporters leading to glutamate-enhanced ATP production in brain mitochondria. PLoS ONE

mahindru ,S.N .,(2004): Food Additives :Characteristics ,Detection and stimation ,McGraw,Hill,New Delhi.

Marletta MA,Nitric oxide synthase.,(1994): Aspects concerning structure and catalysis. Cell .78:927– 930.

Mathew S, Menon PVG and Kurup PA (1985): Effect of administration of vitamin a, ascorbic acid and nicotinamide adenine dinucleotide and flavine adenine nucleotide on severity of

myocardial infarction induced by isoproterenol in rats. Indian Journal of Experimental Biology. 23: 500-504.

McMurray JJ, Ray SG, Abdullah I, Dargie HJ, Morton JJ.,(1992): Plasma endothelin in chronic heart failure. Circulation1992; 85: 1374-1379.

Melanie Humphreys .,(2007): Potassium disturbances and associated electrocardiogram changes. Efvergency Nurse. 15. 5: 28-34.

Menick DR, Renaud L, Buchholz A, Müller JG, Zhou H, Kappler CS, Kubalak SW, Conway SJ, and Xu L .,(2007) :Regulation of Ncx1 gene expression in the normal and hypertrophic heart. Ann N Y Acad Sci 1099:195–203.

Merrill, J. P., Levine, H. D., Somerville, W., And Smith, S., 3rd., (1950):Ann. Intern. Med., 33, 797.

Mescher, A. L.,(2010): Junqueira, s basic histology text and atlas.*12th Ed*: 1-5.

Miguel Arcanjo Areas, Hellen Dea Barros Maluly, Jean Franciesco Vettorazzi, Everardo Magalhães Carneiro.,(2019):Dietary Monosodium Glutamate Does Not Affect the Electrocardiographic Profiles of Diabetic and Nondiabetic Wistar Rats Food and Nutrition Sciences.10:613-625.

Miller AW, Sims JJ, Canavan A, Hsu T, Ujhelyi MR.,(1999): Impaired vagal reflex activity in insulin-resistant rats. J Cardiovasc Pharmacol. 33(5):698-702.

Miyauchi T, Masaki T.,(1999): Pathophysiology of endothelin in the cardiovascular system. *Annu Rev Physiol.*61:391– 415.

Mnaa S., Shaker E. and Azzam A.M. ,(2015): Effects of Heavy Metals and Monosodium L-Glutamate in Food Flavors on Albino Rats. J. Biomol. Res. Ther. 4:3.

Moore KL.,(2003): Congenital malformations due to environmental factors. In: Developing humans. 2nd ed. W. B. Saunders Co. Ltd., Philadelphia. 173-183.

Murray Db, Gardner Jd, Brower Gl, Janicki Js.,(2004): Endothelin-1 Mediates Cardiac Mast Cell Degranulation, Matrix Metalloproteinase Activation, And Myocardial Remodeling In Rats. *Am J Physiol Heart Circ Physiol* 287:H2295-H2299.

Muslih RK., Al-Nimer MS, Al-Zamely OM.,(2002): The study level of malondialdehyde after activation with (H2o2 and CuSO4) and inhibitition of desferoxamine and molsidomine in the serum of patients with acute myocardial infection. Nat. J. Chem. 5: 139-143.

Nayira Abdelbaky, Iman Zaghouly, Nouf Al-Rashead .,(2009): Alpha-lipoic acid and amliodipine ameliorate myocardial infarction induced by isoproterenol in rats.International Journal of Acaemic research .22:615-690.

Nella Ambrosi , Diego Guerrieri 1, Fiorella Caro 1, Francisco Sanchez , Geraldine Haeublein , Domingo Casadei , Claudio Incardona and Eduardo Chuluyan.,(2018): Alpha Lipoic Acid: A Therapeutic Strategy that Tend to Limit the Action of Free Radicals in Transplantation. Int. J. Mol. Sci. 19.102.

Nicolas pilia.,(2012): ECG as atool to estimate potassium and calcium concentrations in the extracellular space. Computing in cardiology.

Ninomiya K.,(1998) :Natural occurrence. Food Review International.14:177-212.

Okon A. Kingsley, Prof. T. W. Jacks3, D. S. Amaza2, Tarfa M. Peters .,(2013): The Effect of Monosodium Glutamate (MSG) on the Gross Weight of the Heart of Albino Rats. Scholars Journal of Applied Medical Sciences . 1(2):44-47.

Oliveira AP, Lunardi CN, Rodrigues GJ, Bendhack LM.,(2009): Relaxation induced by calcium ionophore is impaired in carotid arteries from 2K-1C rats due to failed effect of nitric oxide on the smooth muscle cells. Vascul Pharmacol.50: 153-159.

Oriquat GA, Saleem TH Naik RR Moussa SZ and Al-Gindy RM.,(2012) : A Sub-Chronic Toxicity Study of Mercuric Chloride in the Rat. Jordan Journal of Biological Sciences 5 (2): 141-146.

Ortiz GG, Bitzer-Quintero OK, Zarate CB., (2006): Monosodium glutamate-induced damage in liver and kidney: A morphological and biochemical approach. Biomed Pharmacother. 60: 86-91.

PACHER PA L, JOSEPH S. BECKMAN, AND LUCAS LIAUDET.(2007): Nitric Oxide and Peroxynitrite in Health and Disease. *Physiol Rev* .87: 315–424.

packer L ,H.J.,Tritschiler ,K.,Wessel.,(1997):Neuroprotection by the metabolic antioxidant □-lipoic acid .Free Radic. Biol .Med .22:359-378.

Packer L, Kraemer K, Rimbach G.,(2001): Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition.17: 888-895.

Packer, L., Roy, S., & Sen, C. K., (1997): Alpha lipoic acid: a metabolic antioxidant and potential redox modulator of transcription. Advances in Pharmacology, 38, 79e101.

Panneerselvam.,(2002): Effect of α lipoic acid on neural antioxidant in aged rats .Pharm.Res.42:219-22.

Paravicini TM, and R. M. Touyz.,(2008):NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities," Diabetes care, vol. 31, no. 2, pp. 170–180.

Paul J, L. M. Strijbos, Michael J. Leach, and John Garthwaite.,(1996): Vicious Cycle Involving Na1 Channels, Glutamate Release, and NMDA Receptors Mediates Delayed Neurodegeneration through Nitric Oxide Formation. The Journal of Neuroscience.*16*(16):5004–5013.

Paul S., Mohanan A., Varghese M., Alex M.and Nair H., (2012): Ameliorative effect of α-tocopherol on monosodium glutamate-induced cardiac histological alterations and oxidative stress. Dec.92(15):3002-3006.

Paviovic V, Sarac M .,(2010): The role of ascorbic acid and Monosodium glutamate in thymocyte apoptosis .Bratisl Lek Listy 111:357-360.

Pavlovic V, Pavlovic D, Kocic G., (2009): Ascorbic acid modulates monosodium glutamate induced cytotoxicity in rat thymus. Bratisl Lek Listy. 110: 205-209.

Pavlovic V, Pavlovic D, Kocic G, Sokolovic D, Jevtovic-Stoimenov T., (2007): Effect of monosodium glutamate on oxidative stress and apoptosis in rat thymus. Mol Cell Biochem 303: 161-166.

Peela J, Abdalla M,Abdul-Hadi,Avinaash K.,(2010): Cardiac Biomarkers .the troponin and CK-MB.Ibnosina Journal of Medicine and Biological science .2(5) :190-197.

Priscilla DH and Prince PS (2009): Cardioprotective effect of gallic acid on cardiac troponin-t, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in wistar rats. Chem Biol Interact. 179: 118-124.

Radomski MW, Salas E.,(1995): Nitric oxide-biological mediator, modulator and factor of injury: Its role in the pathogenesis of atherosclerosis. Atherosclerosis.118 (suppl):S69– S80.

Raffaele Marfella, Michelangela Barbieri, Celestino Sardu , Maria Rosaria Rizzo ., (2016): Effects of a-lipoic acid therapy on sympathetic heart innervation in patients with previous experience of transient takotsubo cardiomyopathy. Journal of Cardiology.67 : 153–161.

Reddy VM, Meharg SS, Ritter S.,(1986): Dose-related stimulation of feeding by systemic injections of monosodium glutamate. Physiol Behav. 38(4):465-469.

Reeds PJ, Burrin DG, Stoll B, Jahoor F.,(2000). Intestinal glutamate metabolism. J Nutr. 130:978S-82S.

Ricdijk MA, van Goudoever JB.,(2007): Splanchnic metabolism of ingested amino acids in neonates. Curr Opin Clin Ntitr Metab Care.10:58-62.

Rideout TC, B. Carrier, S. Wen, A. Raslawsky, R. W. Browne, and S. V. Harding, (2015)"Complementary cholesterol-lowering response of a phytosterol/alpha-Lipoic acid combination in obese zucker rats," Journal of Dietary Supplements.

Rogres PP and Blundell JE; Umani and appetite .,(1990): Effects of monosodium glutamate on hunger and food intake in human subject. Phisol. Behav. 486: 801-804.

Sabatine MS, Morrow DA, de Lemos JA ., (2009): Detection of acute changes in circulating troponin in the setting of transient stress testinduced myocardial ischaemia using an ultrasensitive assay: Results from TIMI 35. Eur Heart J. 30: 162-169.

Saladin K.,(2004): Anatomy and Physiology the unity of form and function .Third edition .The McGraw –Hill.company.

Saltiel, A.R. and C.R. Kahn.,(2001): *Insulin signalling and the regulation of glucose and lipid metabolism.* Nature, 2001. **414**(6865): p. 799.

Saravanan G, Ponmurugan P, Sathiyavathi M ., (2013): Cardioprotective activity of amaranthus viridis linn: Effect on serum marker enzymes, cardiac troponin and antioxidant system in experimental myocardial infarcted rats. International Journal of Cardiology. 165: 494- 498.

Sarper Kocaoglu,Ozgur Aktas, Oguzhan Zengi, Azmi Tufan, Feyza Karagoz Guzey.,(2018): Effects Of Alpha Lipoic Acid On Motor Function And Antioxidant Enzyme Activity Of Nerve Tissue After Sciatic Nerve Crush Injury In Rat. Turk Neurosurg 28(5):740-747.

Scott M. Wells, DVM, DACVECC , Meg Sleeper.,(2008): Cardiac troponins. Journal of Veterinary Emergency and Critical Care .18: 235–245.

Şehirli, Ö., Et Al.,(2008): A - Lipoic Acid Protects Against Renal Ischaemia–Reperfusion Injuryin Rats. Clinical And Experimental Pharmacology And Physiology. 35(3): P. 249-255.

Sesso H., Buring J., Norkus E. and Gaziano J., (2005): Plasma lycopene, other carotenoids, and retinol and the risk of cardiovascular disease in men. Am J ClinNutr.,81(5):990–997.

Shannon, M.,(2017): The Endocrine Disrupting Potential of Monosodium Glutamate (MSG) on Secretion of the Glucagon-Like Peptide-1 (GLP-1) Gut Hormone and GLP-1 Receptor Interaction. Toxicology Letters , 265, 97-105.

Sharma A.,(2015): Monosodium glutamate –induced oxidative kidney damaged and possible mechanism . a mini review .J.Biomed .Sci .22:093.

Shimoni, Y., Severson, D. and Ewart, H.S. (2000) Insulin Resistance and the Modulation of Rat Cardiac K(+) Currents. American Journal of Physiology: Heart and Circulatory Physiology, 279, H639-H649.

Signora´ Peres Konrad, Vera Farah,Bruno Rodrigues, Roge´rio Branda˜o Wichi .,(2012): Monosodium glutamate neonatal treatment induces cardiovascular autonomic function changes in rodents. CLINICS .67(10):1209-1214.

Simona Magi, Sara Arcangeli, Pasqualina Castaldo, Annamaria Assunta Nasti.,(2013): Glutamate-Induced ATP Synthesis: Relationship between Plasma Membrane Na1/Ca21 Exchanger and Excitatory Amino Acid Transporters in Brain and Heart Cell Models. Mol Pharmacol 84:603–614.

Singh .U, and I. Jialal.,(2008):Alpha-lipoic acid supplementation and diabetes," Nutrition Reviews, vol. 66, no. 11, pp. 646–657.

Singh K and Ahluwalia P (2012): Effect of monosodium glutamate on lipid peroxidation and certain antioxidant enzymes in cardiac tissue of alcoholic adult male mice. J Cardiovasc Dis Res. 3: 12-18.

Singh K, and Pushpa A., (2005): Alteration in some antioxidant enzymes in cardiac tissue upon monosodium glutamate (MSG) administration to adult male mice. Indian J. Clin. Biochem., 20: 43-46.

Singh U, Jialal I.,(2008): Alpha-lipoic acid supplementation and diabetes. Nutr Rev.66: 646-657.

Smith SE, Lekieffre D, Sowinski P, Meldrum BS., (1993): Cerebroprotective effect of BW619C89 after focal or global cerebral ischaemia in the rat. NeuroReport 4:1339 –1342.

Stoll B, Burrin D, Henry J, Yu H, Jahoor F, Reeds P(1998):Dietary amino acids are the preferential source of hepatic protein synthesis in piglets. J Nutr. 128:1517-1524.

Stoll B, Burrin DO, Henry J. Yu H. Jahoor F, Reeds P.,(1999): Substrate oxidation by the portal drained viscera of fed piglets. Am J Physiol .277:E168-75.

Straus, S.M., (2006): Prolonged QTc Interval and Risk of Sudden Cardiac Death in a Population of Older Adults. Journal of the American College of Cardiology, 47, 362-367.

Suchalatha S. and Shyamala C., (2004):Protective effect of Terminalia chebula against experimental myocardial injury induced by isoproterenol. Int. J. Exp. Biol.42: 174-178.

Suh JH, Wang H, Liu RM.,(2004) :(R)- alpha-lipoic acid reverses the agerelated loss in GSH redox status in post-mitotic tissues: evidence for increased cysteine requirement for GSH synthesis. Arch Biochem Biophys.423(1):126–135.

Surekha R.H., Srikanth B.B.M.V., Jharna P., Ramachandra R.V., Dayasagar R.V. and Jyothy A., (2007): Oxidative stress and total antioxidant status in myocardial infraction. Singapore Med J, 48(2): 137-142.

Suzuki YJ, M.,Tsuchiya, L.,packer.,(1992): Lipoate prevents glucose –induced protein modifications .free Radic .Comm.17:211-217.

Sweatt AJ. Wood M, Suryawan A. Wallin R, Willin gham MC, Hutson SM.,(2004): Branched-chain amino acid catabolism: unique segregation of pathway enzymes in organ systems and peripheral nerves.. Am J Physiol Endocrinol Metab. 286:E64-76.

Szatkowski M, Attwell D., (1994): Triggering and execution of neuronal death in brain ischaemia: 'two phases of glutamate release by different mechanisms. Trends Neurosci 17:359 – 365.

Tahani SS, Al-Azawi and Hayder S, Rwayyih.,(2017) : Protective role of alpha Lipoic acid on some parameters related to metabolic disorders in intact and ovariectomized rabbits. Journal of Entomology and Zoology Studies .5(4): 1281-1285.

Takaoka M, Y. Kobayashi, M. Yuba, M. Ohkita, and Y. Matsumura.,(2001):Effects of α-lipoic acid on deoxycorticosterone acetate-salt-induced hypertension in rats. European Journal of Pharmacology, vol. 424, no. 2, pp. 121–129.

Takaoka, M., (2002):*Protective Effect Of α-LIPOIC Acid Against Ischaemic Acute Renal Failure In Rats.* Clinical and experimental pharmacology and physiology. 29(3): p. 189-194.

Taylor CP, Meldrum BS., (1995): Na1 channels as targets for neuroprotective drugs. Trends Pharmacol Sci 16:309 –316.

Thirunva V Karasu ,A.T., (2004): cardiac lipid and antioxidant status in high fructose rats and the effect of a-lipoic acid .Nutr Metab Cardiovascular Dis.14:351-357.

Thompson PL., (2005) :Corona^ Care Mar^ual. Churrhill Livingstone, London. **Jonathan D Gardner.,**(2014): ECG Diagnosis: The Effect of Ionized Serum Calcium Levels on Electrocardiogram. CLINICAL MEDICINE. 8(1):119-120.

Tietz N.W.,(1999): Text bok of clinical chemistry , 3rd ED . C.A. Burtis, E.R. Ashwood , W>B. Saunders . p.1395-1406,p.1435-1439.

Tonkla Insawang, Carlo Selmi, Ubon Cha'on, Supattra Pethlert.,(2012): Monosodium glutamate (MSG) intake is associated with the prevalence of metabolic syndrome in a rural Thai population. Nutrition & Metabolism . 9:50.

Ulrich Fo¨ Rstermann,William C. Sessa.,(2011): Nitric Oxide Synthases: Regulation And Function PHYSIOLOGY. European Heart Journal .10.1093 .Dis.

Uneyama H, Kawai M, Sekine-Hayakawa Y., (2009): Contribution of umami taste substances in human salivation during meal. J Med Invest. 56 Suppl: 197-204.

Upaganlawar A, Gandhi C and Balaraman R (2009): Effect of green tea and vitamin E combination in isoproterenol induced myocardial infarction in rats. Plant Foods Hum Nutr. 64: 75-80.

Vallianou N, Evangelopoulos A, Koutalas P.,(2009): Alpha-lipoic acid and diabetic neuropathy. *Rev Diabet Stud*. 2009;6(4):230–236.

van den Berg, N.W.E.,(2017) ;Can We Spice up Our Christmas Dinner? Busting the Myth of the "Chinese Restaurant Syndrome". Netherlands Heart Journal ,M. 25, 664-668.

Vanuffelen BE, Van Derzec J, Dekoster BM.,(1998): Biochem J. 330: 719.

Vasdev S, J. Stuckless, and V. Richardson.,(2011):Role of the immune system in hypertension: modulation by dietary antioxidants," International Journal of Angiology, vol. 20, no. 4, pp: 189–212.

Victoria Serhiyenko, Ludmila Serhiyenko, Galyna Suslik, Alexandr Serhiyenko .,(2018): Alpha-lipoic acid: mechanisms of action and beneficial effects in the prevention and treatment of diabetic complications. *MOJ Public Health.*7(4):174–178.

VillarIC,FrancisS,WebbA,HobbsAJ,AhluwaliaA.,(2006):.Novel aspects of endothelium - dependentregulationofvascular tone. KidneyInt.;70:840e53.

Virmani R, Burke AP, Farb A, Kolodgie FD .,(2006) :Pathology of the vulnerable plaque. J Am Coll Cardiol 47: C13-C18.

Viswanatha Swamy Ah, Wangikar U, Koti Bc, Thippeswamy Ah, Ronad Pm, Manjula Dv.,(2011):Cardioprotective Effect Of Ascorbic Acid on doxorubicin-induced myocardial toxicity in rats. IndianJ Pharmacol 43: 507-511.

Von Diemen V, Trindade EN,Trindade MR.,(2006):Experimental model to induce obesity in rats .Acta Cir Bras 21 : 425-429.

Wendy K. Alderton, Chris E. Cooper. Richard G.Knowles.,(2001): Nitric oxide synthases : structure, function and inhibition. Biochem. J. *357*:593-615.

Williams AN,Woessner KM.,(2009): Monosodium glutamate allergy :menace or myth.Clin Exp Allergy 39 :640-646.

Windmueller HG.,(1982): Glutamine utilisation by the small intestine. Adv. Enzymol. Relat. Areas Mol. Biol. 53:201-237.

Wollin SD, Jones PJ.,(2003) :Alpha-lipoic acid and cardiovascular disease. J Nutr. 133: 3327-3330.

Womgcharoen W., Manikandan P., Sumitra M., Aishwarya S., Manohar B., Lokanadam B. and Puvanakrishnan A., (2012): Effects of curcuminoids on frequency of acute myocardial infarction after coronary bypass grafting. Am J Cardiol.110(1):40-4.

Woodrow, P., (2010): An Introduction to Electrocardiogram Interpretation: Part 1. Emergency Nurse , 18, 28-35.

Woods M, Mitchell JA, Wood EG, Barker S, Walcot NR, Rees GM,Warner TD.,(1999): Endothelin-1 is induced by cytokines in human vascular smooth muscle cells: Evidence for intracellular endothelin-converting enzyme. Molecular Pharmacology.55:902–909.

Yamaguchi, S. and Ninomiya, K., (2000): Umami and Food Palatability. The Journal of Nutrition. 130: 921S-926S.

Yang RL, Li W, Shi YH. Le GW.,(2008): Lipoic acid preventshigh-fat diet-induced dyslipidemia and oxidative stress. A microarray analysis. Nutrition. 24(6):582-8.

Yap YG, Camm AJ.,(2003) :Drug induced QT prolongation and torsades de pointes. Heart .89(11):1363–72.

Yasser F, Ali ,Omar S. Desouky Nabila S. Selim , Khairy. M. Ereiba.,(2015): Assessment of the role of a-lipoic acid against theoxidative stress of induced iron overload. Journal of radiation Research and Applied Science .8:26-35.

Yoshida Y.,(1998): Umami taste and traditional seasoning. Food Review International.14(2):213-246.

Young VR, Ajami AM.,(2000): Glutamate: an amino acid of particular distinction. In: International Symposium on Glutamate.130:892S-900S.

Zeinab A. Hassan ,Manar Hamed Arafa ,Wafaa Ibrahim Soliman .,(2014): The effect of Monosodium glutamate on thymic and splenic Immune functions and role of recovery .Biochemical and Histological study.Journal of Cytology and Histology .5:283.

Zhang, Y.,(2011): Amelioration of Lipid Abnormalities by α-Lipoic acid Through Antioxidative and Anti-Inflammatory Effects. Obesity. 19(8): p. 1647-1653.

APPENDIXES

Appendix

Appendix I

Cardiac troponin

Wondfo one step Troponin I whole Blood/Serum / Plasma Test is a rapid immunochrommatographic direct binding test for the visual detection of Troponin I antigen in human whole blood,serum and plasma specimens as an aid in the diagnosis of acute myocardial infarction (AMI) .Wondofo One Step Troponin 1 Whole Blood /Serum/Plasma Test uses double antibody sandwich method.

When the specimen is aadded into the sample well it is absorbed into the device by capillary action , mixes with the antibody –dye conjugate , and flows across the pre-coated membrane.

When the Troponin 1 level in the specimen is at or above the detection limit of of the test , Troponin I bound to the antibody-dye conjugate are captured by

Troponin I antibody immobilized in the Test Region (T) of the device , and this produced a colored test band that indicates appositive result.

When the Troponin I level in the specimen is Zero or below the detection limit of the test , there is not a visible colored band in the in the Test Region (T) of the device . this indicate a negative result.

To serve as a procedure control, a colored line will appear at the control region (C) ,if the test has been performed properly.

Test Procedure

Allow the device and specimen to equilibrate to room temperature (10C -30C)

1.Remove a testing device from the foil pouch by tearing at the notch and place it on a level surface.

2.For Veipuncture Whole Blood testing : Holding a sample dropper vertically , add 3-4 drops (about 80-100ml)of Whole blood specimen into the sample well(with an arrow marked) of the test cassette. For Fingerstick Whole Blood testing: Fill the capillary tube and transfer approximately 80-100ml of fingerstick whole blood specimen into the sample well.

For serum /plasma testing: Holding a sample dropper vertically , add 3-4 drops (about 80-100ml)of serum or plasma to the sample well.

2.Wait for 15 minutes and read results . Do not read results after 30 minutes .

AppendixII

Endothelin 1

This Kit is an Enzyme-Linked Immunosorbent Assay (ELIZA) . The plate has been pre-coated with Rabbit ET-1 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Rabbit ET-1 Antibody is added and binds to Et-1 in the sample. Then Streptavidin –HRP is added and binds to the Biotinylated ET_1 antibody. After incubation un bound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the to the amount of Rabbit ET-1 . The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450nm

Assay procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use . The assay is performed at room temperature.
2. Determine the number of strips required for the assay . Insert the strips in the frames for use. The unused strips should be stored st 2-8 C̊ .
3. Add 50µ1 standard well.. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40µ1 sample to sample wells and then add 10µ1 anti-ET-1 antibody to sample sample wells, then add 50µ1 streptavidin-HRPto sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer . Incubate 60 minutes at 37 C̊ .
5. Remove The sealer and wash the plate 5 times with wash buffer . Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash . For automated washing , aspirate all wells and wash 5 times with wash buffer , ,overfilling wells with wash buffer . Blot the plate onto paper towels or other absorbent material
6. Add 50µ1 substrate solution A to each well and then add 50 µ1 substrate solution b to each well . incubate plate covered with anew sealer for 10 minutes at 37 C̊ in the dark .
7. Add 50µ1 stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to a 450 nm within after adding the stop solution.

Appendix III

Nitric Oxide

PROCEDURES Preparations. Dilute 20x ZnSO4 and 30x NaOH to 1-fold wi ater. The final concentrations are 75 mM ZnSO4 and 55 mM NaOH, respectively. Dilute Activation Buffer by mixing 1 volume of 3 x Activation Buffer with 2 volumes of distilled water. All diluted solutions can be stored at 4°C for 12 months

No	Premix + H_2O + Gly Buffer	Vol (µL)	Nitrite (µM)
1	150µL + 0µL + 50 µL	200	50
2	120µL + 30µL + 50 µL	200	40
3	90µL + 60µL + 50 µL	200	30
4	60µL + 90µL + 50 µL	200	20
5	45µL + 105µL + 50 µL	200	15
6	30µL + 120µL + 50 µL	200	10
7	15µL + 135µL + 50 µL	200	5
8	0µL + 150µL + 50 µL	200	0

. **Procedure using 96-well plate:**

1. Standards. Prepare 600 µL 50 µM Premix by mixing 30 µL 1.0 mM Standard and 570 µL distilled water. Dilute standard in centrifuge tubes as shown below. Add 50 µL Glycine Buffer per tube. Transfer 00 µL diluted standards into wells of a clear-bottom 96-well plate. 1

2. Deproteination is required for serum, plasma and other proteinaceous samples. Mix 100 µL sample with 80 µL 75 mM ZnSO4 in 1.5-mL tubes. If precipitation occurs, centrifuge 5 min at 14,000 rpm. Transfer supernatant to a clean tube containing 120 µL 55 mM NaOH. Pellet protein precipitates again (dilution factor n = 3). Transfer 210 µL supernatant and mix with 70 µL

Glycine Buffer in a 1.5-mL centrifuge tube. If solution remains clear in these steps, deproteination is not required. Directly transfer 210 µL sample (dilution factor n = 1) and mix with 70 µL Glycine Buffer in a 1.5-mL centrifuge tube.

3. Activation of Cd. The number of Cd granules to be used is 3 x the number of samples. Transfer Cd granules in a 50-mL centrifuge tube. Wash Cd three times with water. Remove residual water with a pipet. Add 200 µL diluted 1 x Activation Buffer per granule and incubate 5 min at room temperature. Swirl tube intermittently. Wash three times with water. Activated Cd should be used within 20 min. Note: cadmium is a toxic and expensive metal. Avoid direct contact (wear gloves). About 150 Cd granules are provided that are sufficient for about 50 samples. Used Cd granules should be stored or washed in 0.1 N HCl for at least 5 min. Washed 3 times with water and regenerated using the same activation procedure. Cd granules can be regenerated and used 7 times without loss of activity.

4. Nitrate Reduction. Dry the activated Cd granules on a filter paper (e.g. Kimwipes® EX-L). Add three Cd granules per sample (Step 2) and shake tubes intermittently. Incubate 15 min at room temperature. Transfer 2 x 100 µL samples (duplicate) into wells of the 96-well plate.

5. Assay. Add 50 µL Reagent A to all wells and tap plate lightly to mix. Add 50 µL Reagent B and mix. Incubate 5 min at room temperature. Read OD at 500-570nm (peak 540 nm). Signal is stable for > 60 min.

Appendix IV

Rabbit ATP synthase ,Subunit Alpha , Mitochondrial ELISA KIT

This Kit is an Enzyme -Linked Immunosorbent Assay(ELIZA) . The plate has been pre-coated with Rabbit ATP5A1 antibody. ATP5A1 present in the

sample is added and binds to antibodies coated on the wells . And then biotinylated Rabbit ATP5A1 Antibody is added and binds to ATP5A1 in the sample. Then Streptavidin –HRP is added and binds to the Biotinylated ATP5A1 antibody. After incubation unbound Streptavidin –HRP is washed away during awashing step . Substrate solution is then added and color develops in proportion to the amount of Rabbit ATP5A1 . The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Assay procedure

1. Prepare all reagents, standard solution and samples as instructed. Bring all reagent to room temperature before use. The assay is performed at room temperature.

2. Determine the number of strips require for the assay . Insert the strips in the frames for use. The unused strips should be stored at 2-8 C̊

3. Add 50µ1 standard to standard well. Note : Don't add antibody to standard well because the standard solution contains biotinylated antibody.

4. Add 4oµ1 sample to sample wells and then add 10µ1 anti-Attp5A1 antibody to sample wells, then add 50µ1 streptavidin –HRP to sample wells and standard wells (Not blank control well) . Mix well . Cover the plate with a sealer . Incubate 60 minutes at 37 C̊

5. Remove the sealer and wash the plate 5 times with wash buffer . Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer , overfilling wells with wash buffer . Blot the plate onto paper towels or other absorbent material.

6. Add 50µ1 substrate solution A to each well and then add 50µ1 substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37C̊ in the dark.
7. Add 50µ1 stop solution to each well, the blue color will change into yellow immediately.
8.Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after addig the stop solution.

Appendix V

Rabbit Nitric Oxide Synthsase 3, Endothelial ELIZA KIT

Test principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELIZA) . The plate has been pre-coated with Rabbit NOS3/ENOS present in the sample is added and bind to antibodies coated on the wells. And then biotinylated Rabbit NOS3/ENOS Antibody is added and binds to antibodies coated on thee wells. And then biotinylated Rabbit NOS3/ENOS Antibody is added and binds to NOS3/ENOS in the sample . Then Streptavidin –HRP is added and binds to the Biotinylated NOS3/ENOs antibody .After incubation unbound Streptavidin –HRP is washed away during awashing step. Substrate solution is then added and color develops in proportion to the amount of Rabbit NOS3/ENOS . The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm

Assay Procedure

1. Preare all reagent , standard solutions and samples as instructed . Bring all reagents to room temperature before use ,. The assay is performed at room temperature .

2. Determine the number of strips required for the assay . Insert the strips in the in the frames for use. The unused strips should be stored at 2-8C̊
3. Add 50µ1 standard to standard well. Note : Don't add antibody to standard because the standard solution contains biotinylated antibody.
4. Add 40µ1 sample to sample wells and then add 10 µ1 anti NOS3/ENOS antibody to sample wells, then add 50µ1 streptavidin-HRP to sample wells and standard wells (Not blank control well) .Mix well . Cover the plate with asealer . Incubate 60 minutes at 37C̊
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing , aspirate all wells and wash 5 times with wash buffer , overfilling wells with wash buffer . Blot the plate onto paper towels or other absorbent material .
6. Add 50µ1 Substrate solution A to each well and then add 50µ1 substrate solution B to each well . Incubate plate covered with a new sealer for 10 minutes at 37 C̊ in the dark.
7. Add 50µ1 stop solution to each well , the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution

Appendix VI

Peroxynitrate

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Peroxynitrite Sensor Green stock solution (500X) Add 20 µL of DMSO into the vial of Peroxynitrite Sensor Green and mix well to make 500X stock solution.

Δ Note 20 µL of Peroxynitrite Sensor Green (500X) is enough for 1 plate.

Δ Note Unused Peroxynitrite Sensor Green (500X) can bealiquoted and stored at -20ºC in tightly sealed tubes. Avoid light and freeze-thaw cycles.

5.2 Peroxynitrite Sensor Green working solution (10X)

Add 10 µL of 500X DMSO reconstituted Peroxynitrite Sensor Green (from step 5.1) into 500 µL of Assay Buffer and mix well.

Δ Note The working solution is not stable; prepare it as needed before use.

7. Assay Procedure

 Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

 Assay all standards, controls and samples in duplicate.

7.1 Run the peroxynitrite assay:

EITHER

1. Add 10 µL/well (96-well plate), or 2.5 µL/well (384-well plate) of Peroxynitrite Sensor Green working solution (from Step 5.2) in 90 µL (96-well plate) or 22.5 µL (384-well plate) cell culture per well in the cell plate.

Δ Note It is not necessary to wash cells before staining. It's recommended to stain the cells in full medium.

2. Co-incubate cells with Peroxynitrite Sensor Green (10X) and test compounds in your growth medium / buffer of choice at 37ºC for the desired period of time, protected from light.

Δ Note It is recommended to stain the cells in full medium. However, if the test compounds are serum sensitive, growth medium and serum factors can be aspirated away before staining. Resuspend cells in 1X Hank's salt solution and 20

mM Hepes buffer or the buffer of your choice after aspiration. Alternatively, cells can be stained in serum-free media.

Δ Note: By way of example, we co-incubated RAW 264.7 macrophage cells with 50-200 μM SIN-1 Peroxynitrite Sensor Green in full medium at 37ºC for 1 hour to induce peroxynitrite.

OR

3. Pre-stain cells Peroxynitrite Sensor Green at 37ºC for 1 hour in your growth medium / buffer of choice protected from light (as in 7.1.1) but in the absence of test compounds.
4. Remove the cell medium, then treat the cells with test compounds in the growth medium / buffer of your choice (without Peroxynitrite Sensor Green) at 37ºC for the desired period of time. ab233468 Peroxynitrite Assay Kit (Cell-based) 8

BOTH

5. Measure the fluorescence using a microplate reader at
Ex/Em=490/530 nm or a fluorescence microscope using the FITC filter

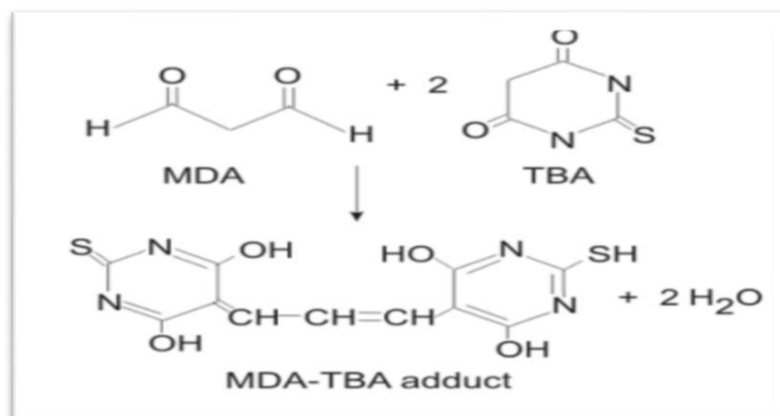
Appendix VII

Estimation of Serum Malondialdehyde (MDA):

Malondialdehyhe was estimated by Thiobarbituric acid (TBA) assay method of Buege & Aust, 1978 on spectrophotometer .

Principle:

This method quantifies lipid peroxides by measuring aldehyde breakdown products of lipid peroxidation. Basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of thiobarbituric acid to form a red MDA-TBA complex which can be measure at 535 nm.



Stock TCA – TBA – HCl Reagent:

It was prepared by dissolving 15% W/V trichloroacetic acid and 0.375% W/V thiobarbituric acid and 0.25N HCl to make 100 ml (2.1 ml of concentrated HCl in 100 ml). This solution was mildly heated to assist in the dissolution of TBA. Dissolved 15 gm TCA and 0.375 mg thiobarbituric acid in 0.25 N HCl and volume was made up to 100 ml with 0.25 N HCl.

Procedure:

To 0.4 ml of serum, 0.6 ml TCA-TBA-HCl reagents were added. It was mixed well and kept in boiling water bath for 10 minutes. After cooling 1.0 ml freshly prepared 1N NaOH solution was added to eliminate centrifugation. This absorbance of pink colour was measured at 535 nm against blank which contained distilled water in place of serum. In blank 0.4 ml distilled water and 0.6 ml TCA-TBA-HCl reagent was mixed and boiled. Blank was always taken.

Calculation:

extinction coefficient of MDA at 535 nm is = 1.56 × 105

MDA concentration = χ / 0.0624 nmol / ml

Appendix VIII

Glutathione(GSH)

Working Assay Mixture preparation

Immediately prior to use ,prepare the working Assay mixture as shown in table

Table 1 preperation is suitable for 48 reaction (100ml/well)

Reagents	Volume
Glutathione Assay Buffer [1X]	5 ml
Glutathione Reductase	8.7 µl
NADPH solution	10 µl

Note NADPH is light sensitive , therefore make the assay buffer in a brown vial or cover the vial with albuminum foil.

Ellman's Working Solution preparation

• Immediately prior to use, add 65 µl Ellman's Reagent stock solution to 2.5 ml of 1X
Glutathione Assay Buffer to make a working solution. You require 50 µl working solution/ well.

NOTE: Ellman's Reagent is light sensitive, therefore make the working solution in abrown vial or cover the vial with aluminum foil

Assay protocol

1. Make dilutions of GSSG in microcentrifuge vials using 400 µM GSSG stock to achieve final concentration of 1 µM, 0.8 µM, 0.6 µM, 0.4 µM, 0.2 µM and 0.1 µM in 1X Glutathione Assay Buffer.

Prepare 1 µM stock of Oxidized Glutathione Standard by adding 2.5 µl of 400 µM Oxidized Glutathione Standard solution to 1 ml of 1 X Glutathione Assay Buffer and mix well.

Tube	Oxidized Glutathione Standard [1µM]	1 X Glutathione Assay Buffer	Final Concentration of Oxidized Glutathione (µM)
A	-	200 µl	0
B	20 µl	180 µl	0.1
C	40 µl	160 µl	0.2
D	80 µl	120 µl	0.4
E	120 µl	80 µl	0.6
F	160 µl	40 µl	0.8
G	200 µl	-	1

NOTE: The detection limit of this assay falls in 0.1-2.5 µM for GSSG and 0.2-5 µM for GSH, so different standard curves can be used.

NOTE: The standards should have the same concentration of Deproteination Reagent as the samples to ensure accurate estimations

2. Aliquot 50 µl GSSG standards into the wells performing in at least duplicate. (See format below).

3. Dilute the 5% Deproteination Solution in the samples to <0.5% by diluting the samples 1:10 with 1X Glutathione Assay Buffer.

NOTE: Lower than 0.5% Deproteination Reagent in sample is acceptable for the assay, however >0.5% Deproteination Reagent is not recommended as it may interfere with the assay. The standards should have the same concentration of Deproteination Reagent as the samples to ensure accurate estimations.

4. Aliquot 50 µl samples into the wells performing in at least duplicate. (See format

below).

5. Add 100 µl of freshly prepared working assay mixture per well..

6. Incubate the plate at room temperature for 5 minutes.

7. Rapidly add 50 µl of freshly prepared Ellman's Reagent working stock solution per
well and mix several times by pipetting up and down.

8. Cover the plate with aluminum foil or incubate plate in dark on shaker until absorbance is checked. For kinetic method absorbance at 0 minute is also recorded

9. Glutathione concentration can be determined by endpoint method or by kinetic method

End point method: Read the plate at 405-415 nm, 25 minutes after addition of Ellman's Reagent.

Kinetic method: Read the plate at 405-415 nm at 5 minutes interval after addition of substrate for 30 minutes

Appendix IX

Mineralx

Sodium

Assay Principe

The present method is based on reaction of sodium with a selective chromogen producing a chromophore whose absorbance varies directlyas the concentration of sodium in the test specimen

Reagents

Reagent(R) Color Reagent

Chromogen	0.03gm/l
EDTA	25mmol/l
Dimethyl Sulfoxide	75mmol/l
Preservatives	0.05%
Antifoam	0.01%
Standard (S)Sodium	150mEq/l

Procedure

Reagents	**Blank**	**STD**	**Sample**
Reagent(R)	1ml	1ml	1ml
Standard	------	10µL	------
Sample	------	------	10µL
Mix well, let stand for 5minute at R.T. then read absorbances , A standared and A sample against Reagent Blank at 630 nm.			

Potaasium

Assay Principle

At an alkaline PH potassium ions and TPB from a tubrid emulsion, the increase of which can be measured quantitatively in a photometer at 578nm. The increase of

the absorbance (A) is directly propotional to the concenteration of potassium in the sample.

Reagent

Reagent R NaOH 0.50mol/l

TPB-Na 240 mmol/l

Irritant (Xi) : R36/38: Irritating to eyes and skin .S26: in Case of contact with eyes/ , Rinse immediately with plenty of water and seek medical advice. S37/39 : Wear suitable gloves and eye/face protection.

Standard Potassium 5.00 mmol/l

Procedure

Reagents	**Blank**	**STD**	**Sample**
Reagent(R)	1ml	1ml	1ml
Standard	------	20µL	------
Sample	------	------	20µL
Mix, incubate for 3minutes at 37 C̊ or 5minutes at 25 C̊ , Mix again thoroughly and read absorbance of sample (Asample) and standard (Astandard)against blank.			

Calculation

Serum Potassium Conc.(mmol/l)=

Expected values

Serum 3.6-5.5 mmol/l

Plasma 4.0-4.8 mmol/l

Calcium

Assay series

	Blank	**STD**	**Sample**
Sample	------	------	25 µl
Distilled Water	25 µl	------	------
Standard	------	25 µl	------
Working Reagent	1.0 ml	1.0 ml	1.0 ml
Mix, read absorbance of the sample (Asample)and standard (Astandard) against the reagent blank after 5 to 50 minutes. Although the absorbance may be read at any time after 5 to 50 minutes , the time interval from sample addition to read time must be exacty the same for Standard/Control and Sample.			

Procedure

Select Calcium CPC in the Run Test Screen and carry out a water blank as instructed

Pipette into cuvette:

	Blank SO	**STD SI**	**Sample**
dd H2o	12.5 µl	-	-
Sample	-	-	12.5 µl
Standard	-	12.5 µl	-
Reagent	500 µl	500 µl	500 µl
Mix, incubate for 5 min at 25, 30, or 37̊ C Insert the cuvette into the Rx Monza flowcell holder and press Read .			

Appendix X

Estimation of serum cholesterol concentration (mg/dl):

Principle:

Easter of cholesterol+H2O $Chol.\ estease$ Cholesterol + Fatty acids

Cholesterol +O2 $Chol.\ oxidase$ Cholest-4-en-one+H2O2

H2O+4-Aminophenazone + phenol $peroxidase$ Quinonimine

Reagent:

Reagent (1) Buffer solution: pipes PH 6.9 mmol/L, phenol 26 mmol/L

Reagent (2) vial of enzyme: cholesterol oxidase 300 U/L, peroxidase 1250 U/L, cholesterol esterase 300 U/L, 4-aminophenazone 0.4 mmol/L

Reagent (3): cholesterol standard 200 mg/dl

1. Manual procedure: Cholesterol concentration in serum samples was measured according to the following

a. Reagent and serum samples were brought to room temperature

b. Serum sample, blank and standard were treated as follow:

c. Tube contents were mixed and left to stand for 5 minutes at 37 °C before reading

d. the absorbance of the standard was measured and sample was read via spectrophotometer at wavelength 505 nm against the blank

Tubes	Blank	Standard	Sample
Cholesterol standard (s)	-	10 ml	-
Sample	-	-	10 ml
Working Reagent	1ml	1ml	1ml

Calculation:

Result were calculated according to the following equation:

Total Cholesterol concentration = (O.D sample)/ (O.D/ standard)× nn = 200 mg/dl

Estimation of serum triglyceride concentration (mg/dl):

Principle:

Triglyceride $\xrightarrow{lipoprotein\ lipase}$ Glycerol + fatty acid

Glycerol + ATP $\xrightarrow{Glycerol\ kinase, Mg^{++}}$ Glycerol-3-phosphate+ADP

Glycerol-3-P+O_2 $\xrightarrow{3-G-P-oxidase}$ Dihydroxyacetone ne-p+H_2O

H2O2+4-Aminophenazone+p+Chlorophenol $\xrightarrow{peroxidase}$ Quinonimine+ H_2O

Reagent:

Reagent (1) buffer solution: pipes buffer PH 7.2, 50 mm0l/L, p- chlorophenol 2 mmol/L

Reagent (2) Enzyme: lipoprotein lipase 150 000 U/I, glycerol kinase 800 U/U/I, glycerol-3-phosphate oxidase 4000 U/I, peroxidase 440 U/I, 4-aminophenazone 0.7 mmol/L, ATP 0.3 mmol/L.

Reagent (3) triglyceride Standard (S): Glycerol 200mg/dl.

Procedure:

Triglyceride concentration in serum samples was measured according to the

following:

a. Wave length/filter. 505nm (Hg546nm)/green

b. Temperature 37°C/R. T

c. Light path 1 cm

Pipette into clean dry test tubes labelled as Blank (B), standard (S), and Test(T). Mix well and incubated at 37°C for 5 min or at R. T (25°C) for 15min. measure the absorbance of the standard

Calculation:

Results were calculated according to the following equation:

Triglyceride concentration mg/dl = (O.D sample) / (O.D standard) × n = 200 mg/dl

Additive sequence	Blank	Standard	Test

Working reagent	1.0	1.0	1.0
Distilled water	0.01	-	-
Triglyceride standard	- Appendix	0.01	-
Sample	-	-	0.01

Estimation of serum HDL-Cholesterol concentration (mg/dl):

Principle:

Cholesterol esters + H_2O *Chol.esterase* Cholesterol + fatty acid

Cholesterol +½O_2 + H_2O *Chol.oxidase* Cholestenone + H_2O_2

2 H_2O_2 + 4-Aminoantipyrine + DCFS *peroxidase* Quinoneimine + 4H_2O

Reagent

Reagent (1) Good's buffer (pH 6.6)100 mmol/l, cholesterol esterase 1400 U/l, cholesterol oxidase 800 U/l, catalase 600 kU/l, N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS) 0.6 mmol/l

Reagent (2) Good's buffer (pH 7.0) 100 mmol/l peroxidase 3 kU/l 4–aminoantipyrine (4-AA) 4 mmol/L

Tubes	Blank	Standard	Sample
Distilled	50 ml	-	-

water			
Cholesterol standard (S)	-	50 ml	-
Sample supernatant	-	-	50 ml
Reagent	1.0ml	0ml	1ml

Procedure:

HDL-Cholesterol concentration in serum sample was measured according to the following steps: serum sample 40 – 60 mg/dl 1.04 1.55mmol/l, wavelength 600 nm,

temperature 37°C CORMAY HDL DIRECT is intended for automated analysers.

a. Reagent (A, B) and serum sample were brought to room temperature.

b. Serum sample, blank and standard were treated as followed:

c. 0.2 ml of sample was mixed with 0.5 ml of reagent (A) in centrifuge tube and let stand for 10 minute at room temperature.

d. Centrifuged at a minimum of 4000 r.p.m. for 10 minutes.

e. The temperature was collected carefully.

f. Sample supernatant, blank, standard and reagent (B)were treated as follows:

g. Tubes contents were mixed thoroughly and incubated for 10 minute at 37°C.

h. the absorbance (A) of the standard was measured and sample was read via spectrophotometerat wave length 500 nm against the blank.

Calculation: results were calculated according to the following equation:

HDL-cholesterol concentration in the sample (mg/dl) = (Absorbance of the sample/Absorbance of standard) × concentration of standard × sample dilution factor (1.7).

Estimation of serum LDL-Cholesterol concentration (mg/dl):

Principle:

Cholesterol ester $\xrightarrow{chol.esterase}$ chol. + fatty acid

Cholesterol + O_2 $\xrightarrow{Chol.oxidase}$ chol. H_2O_2

$2H_2O_2$ $\xrightarrow{catalase}$ $H_2O + O_2$

Reagent:

Reagent (1) Good's buffer (pH 7,0) 50 mmol/l, cholesterol esterase 600 U/l, cholesterol oxidase 500 U/l, catalase 1200 kU/l, ascorbate oxidase 3 kU/l, TOOS [N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline] 2.0 mmol/l

Reagent (2) Good's buffer (pH 7,0) 50 mmol/l, peroxidase 5 kU/l, 4–aminoantipyrine (4-AA) 4 mmol/l.

Procedure:

wavelength 600 nm, temperature 37°C, CORMAY LDL DIRECT is intended for automated analysers. serum/plasma < 100 mg/dl < 2.59 mmol/l.

As LDL cholesterol is affected by a number of factors such as smoking, exercise, hormones, age and sex, each laboratory should

establish its own reference ranges for local population.

Appendix XI

Histological study

Histological Technique(E & H) stain

The Heart and aorta of each animal were quickly removed and rapidly weighed then prepared for histological study according to Mescher method,(2010) with aid of the light microscope as the

following steps:

* **Fixation**

The specimen fixated in the formalin 10 % for 24 – 48 hours.

* **Washing and dehydration**

After fixation the specimens washed with water to remove the fixative in order to avoid the interaction between the fixative and staining materials used later. By dehydration the water had been completely extracted from fragments by bathing them successively in a graded series of of ethanol and water (70 %, 80 %, 90 %, and 100 % ethanol).

* **Clearing**

Bathing the dehydrated fragments in solvent (xylene) for 30 – 60 minutes, this step was repeated 3 times .As the tissues clearing ,they generally became transparent.

* **Infiltration and embedding**

Once the tissue fragments were impregnated with the solvent, they were placed in melted paraffin in an oven, typically at 52℃. The heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin

* **Sectioning**

After holdes from the oven, the specimen let at room temperature to be solid and removed from their containers in order to sectioning they were put in the rotary

microtome and were sliced by the microtome, a steel blade into sections 5 micrometers thick . The sections were floated on water bath (50 – 55 o C) , then transferred into glass slides coated with Mayers albumin as adhesive substance and left to dry.

* **Staining**

The histological sections of the studied organs were stained with Hematoxylin - Eosin stain.

Trichrome stain (modified masson's) procedure

The procedure was done according to Trichrome Stain Kit
(Modified Masson's) ScyTek Laboratories, Inc./ U.S.A.

Procedure

1.Mordant in Bouins solution , microwave 1 minute , allow to strand 15 minute
2.wash in running tap water to remove the picric acid ,5 minutes.
3.weigerts working hematoxylin ,10 minute.
4.Blue in running tap water for 5minute , rinse in distilled water.
5.Biebrich scarlet for 5 minute
6.Rinse in distilled water
7.phosphotungistic/phosphomolyboic acid for 10 minute . discard solution
8.Transfer directly into Anilline blue for 5 minutes.
9.Rinse in distilled water
10. 1% Acetic acid for 1minute ,discard solution ,rinse in distilled water .
11.Dehydrate , clear, and coverslip.
Conventional method :Mordant in Bouins solution ,60 C̊ for 1 hour.

الخلاصة

صممت هذه الدراسة للكشف عن الدور الوقائي لحامض الفا لايبوك ALA ضد تلف غلوتامات الصوديوم الاحادية MSG الذي يؤدي الى احداث تلف القلب والاوعية الدموية في ذكور الارانب , تم تقدير بعض المعايير القلبية في المصل على سبيل المثال (Cardiac troponin I, Endothelin -1) وبعض المؤشرات الحيوية للقلب NO, وفعالية بعض الانزيمات(ATP synthase , NO synthase) , وتركيز المعادن [K],[Na] and Ca]], وبعض معايير الأكسدة (ONOO,MDA) . ومضادات الاكسدة (GSH) , والمؤشرات الدهنية ,(VLDL-C,LDL-C,HDL-C,TG ,TC), وقياس اطوال موجات القلب الكهربائية (P,QRS,T,ST,QT) وتم اجراء فحص لأنسجة القلب والابهر .

قسمت عشوائيا 32 من ذكور الارانب البالغة الى اربع مجاميع (8/مجموعة) .استلمت المجموعة الاولى GI مياه طبيعية كمجموعة سيطرة .استلمت المجموعة الثانيةGII التجريع عن طريق الفم (8ملغم/كغم من وزن الجسم) من غلوتامات الصوديوم الاحادية . استلمت المجموعة الثالثة(GIII) (60ملغم /كغم من وزن الجسم) من حامض الفا لايبوك , استلمت المجموعة الرابعة GIV(8ملغم/كغم من وزن الجسم من غلوتامات الصوديوم الاحادية و 60 ملغم /كغم من وزن الجسم من حامض الفا لايبوك) لمدة 10 اسابيع .

جمعت عينات الدم بتقنية طعنة القلب بعد مرور 10 اسابيع من التجربة لتقييم امكانية شفاء التلف الحاصل في القلب والاوعية الدموية (الابهر) الناتج من اعطاء MSG غلوتامات الصوديوم الاحادية

تشير نتائج دراستنا الى وجود زيادة معنوية ($p<0.05$) في مستوى المصل في(ONOO ,MDA) والنوع الضار للدهون(VLDL_C,LDL_C,TC ,TG) مع زيادة معنوية في مستوى مصل الدم (cardiac troponin I,endothelin 1) , وزيادة معنوية في فعالية (ATP synthase) في مجموعة الحيوانات وزيادة معنوية في تركيز [Na] , كما كشفت نتائجنا زيادة معنوية ($p<0.05$) في وزن الجسم والقلب في حين اظهرت انخفاض معنوي في تركيز المستوى المعنوي (NO, GSH ,HDL),واظهرت موجات القلب الكهربائي في الدراسة الحالية زيادة معنوية في(P,QRS, T waves),(ST,QT intervals) , في المجموعة الثانية GII مقارنة مع(GI, GIII,GIV). ويتم التجريع عن طريق الفم من 60ملغم /كغم يوميا من حامض الفا لايبوك في GIII واعطاءALA و MSG معا في المجموعة GIV

, اظهرت النتيجة تحسن الشفاء في القلب والابهر من خلال انخفاض المستوى المعنوي ($p<0.05$) في مستوى مصل الدم (MDA,,ONOO)والدهون السيئة (VLDL-C,LDL_C,TC <TG) ايضا زيادة كبيرة في المستوى المعنوي ($p<0.05$) في تركيز مستوى المصل في (GSH,NO) في مجموعة الحيوانات GIII مقارنة مع مجموعاتGII.

وقد اظهرت نتيجة الدراسة لدينا انخفاض في المستوى المعنوي($p<0.05$) في المصل (cardiac troponin I,endothelin 1, ATP synthase) للمجموعات GIII,GIV مقارنة مع مجموعة GII.

واظهرت الدراسة الدور الوقائي لحامض الفا لايبوك في انخفاض المستوى المعنوي ($p<0.05$) في موجات القلب (P, QRS ,T waves),(ST ,QT intervals) للمجموعات GIV,GIIIمقارنة معGII

و اظهر الفحص النسجي المرضي في مجموعة الحيوانات التي جرعت MSG في القلب والابهر (الالتهاب , التليف , وانتشار الخلايا الالتهابية والنزيف والتنخر) بالإضافة الى تثخن في الشريان الابهر واظهرت الدراسة الدور الوقائي لحامض الفا لايبوك على نسيج القلب والابهر في مجموعة الحيوانات

(GIII,GIV).

نستنتج من الدراسة الحالية ان الأطعمة التي تحتوي على غلوتامات الصوديوم الاحادية الزائدة يمكن تحصينها عن طريق استخدام حامض الفا لايبوك 60 ملغم/كغم من وزن الجسم لتجنب الجسم من اثارها الضارة خاصة في امراض القلب.

الخلاصة

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء/كلية الطب البيطري
فرع الفسلجة والكيمياء الحياتية والادوية

التاثير الوقائي لحامض الفا لايبوك على جهاز القلب الوعائي في ذكور الارانب التي تتعرض لغلوتامات الصوديوم الاحادية

رسالة مقدمة الى
مجلس كلية الطب البيطري – وهي جزء من متطلبات نيل
درجة الماجستيرفي علوم الطب البيطري /الفسلجة

من قبل
ايمان محمد هيل
بكالوريوس طب وجراحة بيطريه
طب بيطري/جامعة كربلاء2013

بأشراف

ا.د.وفاق جبوري البازي
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