"GREEN SYNTHESIS AND CARDIOPROTECTIVE ACTIVITY OF *NYCTANTHUS ARBOR-TRISTIS* ZNO NANOPARTICLE AGAINST ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS"

A Dissertation submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

Chennai-600032

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY IN PHARMACOLOGY

Submitted by

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This work is original and has not been submitted earlier for the award of any other Degree or Diploma of this or any other university.

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Place: **Mr. P.SUDHAKAR, M. Pharm.,**

DECLARATION

This is to certify that the Dissertation entitled "**Green synthesis and Cardioprotective Activity of *Nyctanthus arbor-tristis* ZnO Nanoparticles against Isoproterenol Induced Myocardial Infarction in Rats**" submitted to **The Tamil Nadu Dr. M.G.R. Medical University,** Chennai, is a bonafide project work carried out myself **S.HARIPRIYA (Reg. No: 261625551)** in the Department of Pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode for the partial fulfillment for the degree of Master of Pharmacy under the guidance and direct supervision of **Mr. P.SUDHAKAR, M. Pharm.,** in the Department of Pharmacology during the academic year of 2017-2018.

Hereby I declare that this work embedded in the thesis is original and not submitted in part or full for any other degree of this or any other university.

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EVALUATION CERTIFICATE

This is to certify that the Dissertation entitled "**Green synthesis and Cardioprotective Activity of *Nyctanthus arbor-tristis* ZnO Nanoparticles against Isoproterenol Induced Myocardial Infarction in Rats** "submitted to **The Tamil Nadu Dr. M.G.R. Medical University,** Chennai, in partial fulfillment for the degree of Master of Pharmacy. This was carried out by **S.HARI PRIYA (Reg. No: 261525552)** under the guidance and direct supervision of **Mr. P.SUDHAKAR, M. Pharm.,** in the Department of Pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode for the during the academic year of 2016-2017.

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ABSTRACT

Title	:	**Green synthesis and Cardioprotective Activity of *Nyctanthusarbor-tristis* ZnO Nanoparticles against Isoproterenol Induced Myocardial Infarction in Rats.**
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Year	:	2017-2018

Aim:

Myocardial infarction is a life-threatening condition that occurs when blood flow to the heart is abruptly cut off, thereby causing tissue damage. The leaves of *Nyctanthus arbor-tristis* mainly used in ayurvedic, siddha, unani. Because of the presence of more potent oleanolic acid in the leaves, the hydroalcoholic extract was therefore investigated for its cardioprotective activity in Nano-form. The present study was therefore aimed for Green synthesis and Cardioprotective activity of *Nyctanthus arbor-tristis* ZnO Nanoparticle against Isoproterenol Induced Myocardial Infarction in Rats.

Materials and methods

Thirty Albino Wistar male Rats weighing 200-300 gm were randomly assigned to iver groups, each group containing 6 animals. **Group I** Control group- Received distilled water p.o for 14 days, **Group II** Negative control group- Received Isoproterenol (85 mg/kg) p.o, **Group III** Standard group - Received Propranolol (10 mg/kg/day), p.o, **Group IV** – Received HAE-NAT (500 mg/kg/day) p.o., **Group V-** Received ZnO NP-NAT (30 mg/kg) p.o. Myocardial Infarction was induced by intra peritoneal injection of Isoproterenol 85 mg/kg in two consecutive dose on 14^{th} and 15^{th} day. The Nanoparticle was synthesized and evaluated against Isoproterenol induced MI by monitoring serum cardiac activity markers like AST,ALP, ALT,LDH,CK-MB. Changes were finally confirmed by Histopathological studies.

Results:

In this study ,ZnO NP against isoproterenol -induced animals exhibited significant increased in serum total cholesterol, TG, LDL level and decrease in HDL. And also serum cardiac activity markers CK -MB, LDH, ALT, AST and ALP were elevated, which were reserved to near normal levels in the treatment of ZnO NP in NAT.

Conclusion:

It might be concluded Hydroalcoholic extract of *Nyctanthus arbor tristis* in cardioprotective activity which might be aid to reduce the myocardial infarction, Cardioprotective effect of ZnO NP was proved by reduction in cardiac marker enzymes, altered lipid profile and histopathological studies

ABBREVIATIONS

MI	Myocardial Infarction
HAE	Hydroalcoholic Extract
CPCSEA	Committee for the Purpose of Control and Supervision on Experiments on Animal
CVS	Cardio vascular system
ISO	Isoproterenol
ZnO	Zinc oxide Nanoparticles
NAT	Nyctanthus arbor-tristis
AST	Aminotransferase
NP	Nanoparticles
ALP	Alkaline phosphatase
CK-MB	Creatinine phosphokinase
LDH	Lactate dehydrogenase
TG	Triglycerides
TC	Total cholesterol
LDL	Low density lipoprotein
HDL	High density lipoprotein

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CHAPTER - 1

INTRODUCTION

Myocardial infarction (MI) or heart attack is one of the leading causes of death all over the world. It is caused due to an interruption in blood supply via the coronary circulation to any part of myocardium, resulting in myocardial necrosis[1]. Consequences of MI include hyperlipidemia, peroxidation of membrane lipids and loss of plasma membrane integrity. The pathogenesis of cardiac damage involves cell apoptosis, which is mainly influenced by oxidative-ROS production. Reactive oxygen species (ROS) play a critical role in the pathogenesis of cardiovascular injury associated with circulatory disturbance[2].

Despite major therapeutic advances, MI remains the major cause of death in india and increased mortality due to CVD is expected to be double by 2020[3]. Hence Myocardial cell protection and prevention of cell ischemia/necrosis have been therapeutic targets for a long time. The allopathic medicines currently used to treat myocardial infarction have many side effects. Hence new therapies are needed to treat myocardial damage limiting its adverse effects and economical costs.

The model of Isoproterenol-induced myocardial necrosis has the mechanism of generating ROS causing lipid peroxidation damage to the proteins due to production of carbonyl derivatives[4]. A disparity between the oxygen requirement of the myocardium and the ability of the coronary artery to meet it results in the ischemic necrosis of heart muscle. The pathophysiological changes following ISO administration are comparable to those taking place in human MI[5]. Hence this model is most widely used in order to study the beneficial effects of various herbal drugs on cardiac function[6].

Triterpenoids exists widely in nature and are the one of the major components of many traditional medicinal herbs. Oleanolic acid (OA) is a triterpenoid compound that exists widely in food and herbs[7]. It has variety of biological effects, such anti-oxidants[8], antifungal, anti-inflammatory, anti-hyperlipdemia, hepatoprotective, tumor prevention, immunomodulatory[9], anti-HIV , anti-arrhythmic and cardiotonic[10]. Due to its anti-oxidant, anti-hyperlipedemic, antiarrhythmic, and cardiotonic effects, it will provide an accessible and cheap traditional medicine source for treatment of myocardial ischemia in developing countries. Hence current attention has been focused on phytoconstituents (OA) derived from plant species as potential therapeutic agents in the prevention and management of cardiovascular disease.

Nyctanthes, also known as Harsingar, is an important member of Ayurveda, the traditional Indian medicine science. It is blessed with a diverse spectrum of medicinal properties, such as anti-helminthic, antimicrobial, antiviral, antileishmania , anti-allergic, anti-diabetic and anti-cancerous. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic[11]. The phytochemical characterization of *Nyctanthus arbour-tristis* reveals the presence of robust bioactive Triterpenoid compound, Oleanolic acid[12]. Hence this boosts up in investigating the effect of leaves on Isoproterenol-induced cardiac injury. Previously reported articles on Oleonolic acid and myocardial damage prompted our further research to deal with Cardioprotective activity of the plant.

Nanoparticles hold extraordinary and attractive properties due to their small sizes, large surface area, free hanging bonds and superior reactivity. Nowadays, nanotechnology has a vast range of application in diagnosis, drug delivery, food industry, paints, electronics, sports, environmental cleanup, cosmetics, and sunscreens. Green synthesis approaches of herbal extracts are gaining interest

towards treatment of various diseases. Recently, plants and their extracts based nanoparticles synthesis were considered to be the best techniques because of easy availability, mass production and eco-friendly process. Zinc (Zn) an essential micronutrient that exhibits antioxidant properties and protects cardiac cells against different oxidative stressors[13]. According to some previous studies, Zinc oxide nanoparticles (ZnO NPs) are found to be non-toxic, biosafe, biocompatible making them an ideal candidate for biological applications.

The plant *Nyctanthes* extracts have been reported to yield gold, silver and titanium dioxide Nanoparticles. Two majorly researched substrates for biosynthesis of ZnONPs are zinc acetate and zinc nitrate . This is, to the best of our knowledge, the first study reporting synthesis of zinc oxide Nanoparticles using leaf extract of *Nyctanthes arbor-tristis* and zinc acetate. The present study was therefore aimed to determine the Cardioprotective effect of NA-ZnO NP against Isoproterenol induced Myocardial Infarction in Rats.

CHAPTER – 2

LITERATURE REVIEW

2.1. MYOCARDIAL INFARCTION

A myocardial infarction is a life-threatening condition that occurs when blood flow to the heart is abruptly cut off, causing tissue damage. This is usually the result of a blockage in one or more of the coronary arteries.

A blockage can develop due to a buildup of plaque, a substance mostly made of fat, cholesterol, and cellular waste products[14] .Coronary atherosclerosis is a chronic disease with stable and unstable periods. During unstable periods with activated inflammation in the vascular wall, patients may develop a myocardial infarction. Myocardial infarction may be a minor event in a lifelong chronic disease, it may even go undetected, but it may also be a major catastrophic event leading to sudden death or severe hemodynamic deterioration. A myocardial infarction may be the first manifestation of coronary artery disease, or it may occur, repeatedly, in patients with established diseases[15].

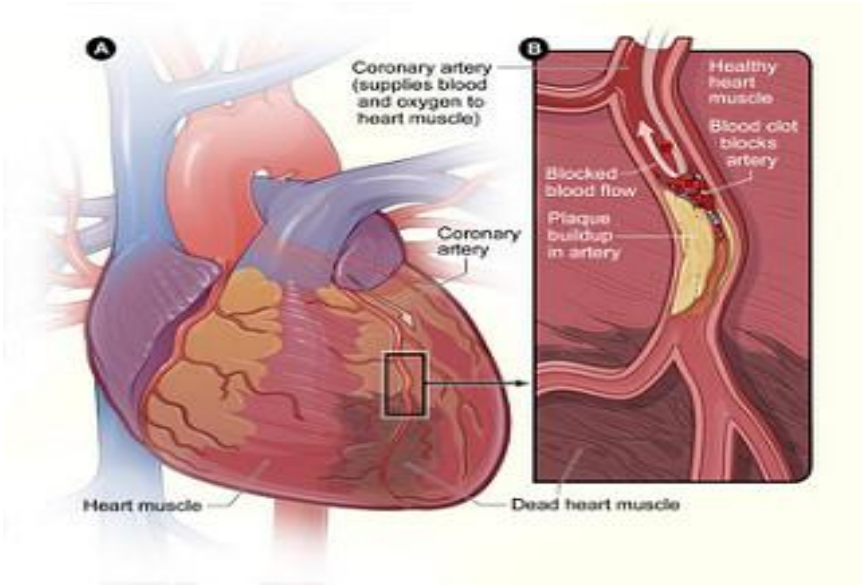


Figure 1: Pathophysiology of Myocardial Infarction

2.1.1. SYMPTOMS

The symptoms can be quite varied. The most common symptoms of a heart attack include,

- Pressure or tightness in the chest
- Pain in the chest, back, jaw, shoulder and other areas of the upper body that lasts more than a few minutes or that goes away and comes back.
- Shortness of breath
- Sweating
- Nausea
- Vomiting
- Anxiety
- Cough
- Dizziness
- Fast heart rate

It's important to note that not all people who have heart attacks experience the same symptoms or the same severity of symptoms. Chest pain is the most commonly reported symptoms among both women and men.

2.1.2. PATHOPHYSIOLOGY

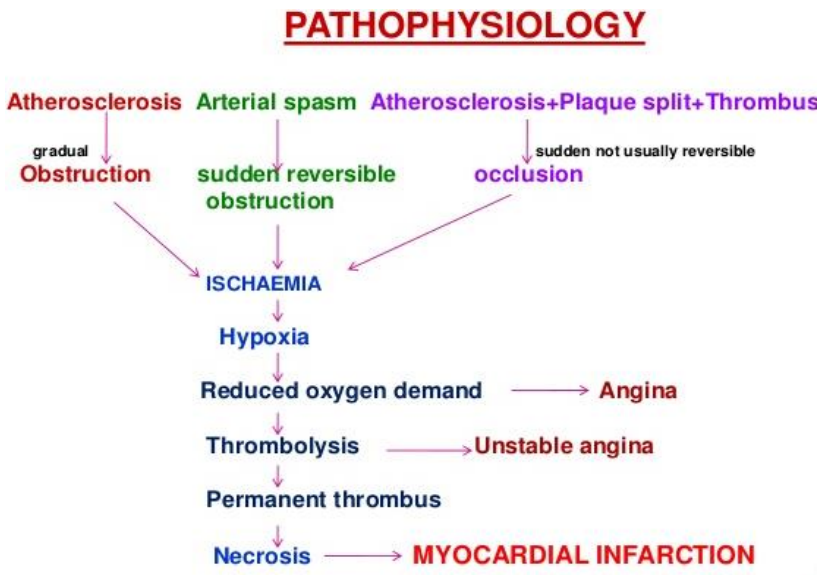


Figure 2: Schematic representation of MI

Most myocardial infarctions are caused by a disruption in the vascular endothelium associated with an unstable atherosclerotic plaque that stimulates the formation of an intracoronary thrombus, which results in coronary artery blood flow occlusion. If such an occlusion persists for more than 20 minutes, irreversible myocardial cell damage and cell death will occur. The two primary characteristics of the clinically symptomatic atherosclerotic plaque are a fibro muscular cap and an underlying lipid-rich core. Plaque erosion can occur, because of the actions of matrix metallo proteases and the release of other collagenases and proteases in the plaque, which result in thinning of the overlying fibro muscular cap. The action of proteases, in addition to hemodynamic forces applied to the arterial segment, can lead to a disruption of the endothelium and fissuring or rupture of the fibro muscular

cap. The loss of structural stability of a plaque often occurs at the juncture of the fibro muscular cap and the vessel wall, a site otherwise known as the shoulder region. Disruption of the endothelial surface can cause the formation of thrombus via platelet-mediated activation of the coagulation cascade. If a thrombus is large enough to occlude coronary blood flow, an MI can result.

The death of myocardial cells first occurs in the area of myocardium, most distal to the arterial blood supply: the endocardium. As the duration of the occlusion increases, the area of myocardial cell death enlarges, extending from the endocardium to the myocardium and ultimately to the epicardium. The area of myocardial cell death then spreads laterally to areas of watershed or collateral perfusion. Generally, after a 6 to 8 hour period of coronary occlusion, most of the distal myocardium has died. The extent of myocardial cell death defines the magnitude of the MI. If blood flow can be restored to at-risk myocardium, more heart muscle can be saved from irreversible damage or death.

The severity of an MI depends on three factors:

1) The level of the occlusion in the coronary artery,

2) The length of time of the occlusion,

3) The presence or absence of collateral circulation.

Generally, the more proximal the coronary occlusion, the more extensive the amount of myocardium that will be at risk of necrosis. The larger the myocardial infarction, the greater the chance of death because of a mechanical complication or pump failure. The longer the period of vessel occlusion, the greater the chances of

irreversible myocardial damage distal to the occlusion. Myocardial necrosis begins at approximately 30 minutes after coronary occlusion. Classic acute MI with extensive damage occurs when the perfusion of myocardium is reduced severely below its needs for an extended interval (usually atleast 2 to 4 hours) causing profound ischemia and resulting in permanent loss of function of large regions in which cell death has occurred[16].

2.1.3. CLASSIFICATION

Myocardial infarctions are generally classified into ST elevation MI (STEMI) and non-ST elevation MI (NSTEMI). STEMI is the combination of symptoms related to poor oxygenation of the heart with elevation of the ST segments on the electrocardiogram followed by an increase in proteins in the blood related to heart muscle's death. They make up about 25 to 40 percent of cases.

The phrase "heart attack" is often used non-specifically to refer to a myocardial infarction and to sudden cardiac death. An MI is different from cardiac arrest, but can cause cardiac arrest, where the heart is not contracting at all or so poorly that all vital organs cease to function. It is also distinct from heart failure, in which the pumping action of the heart is impaired. However, an MI may lead to heart failure.

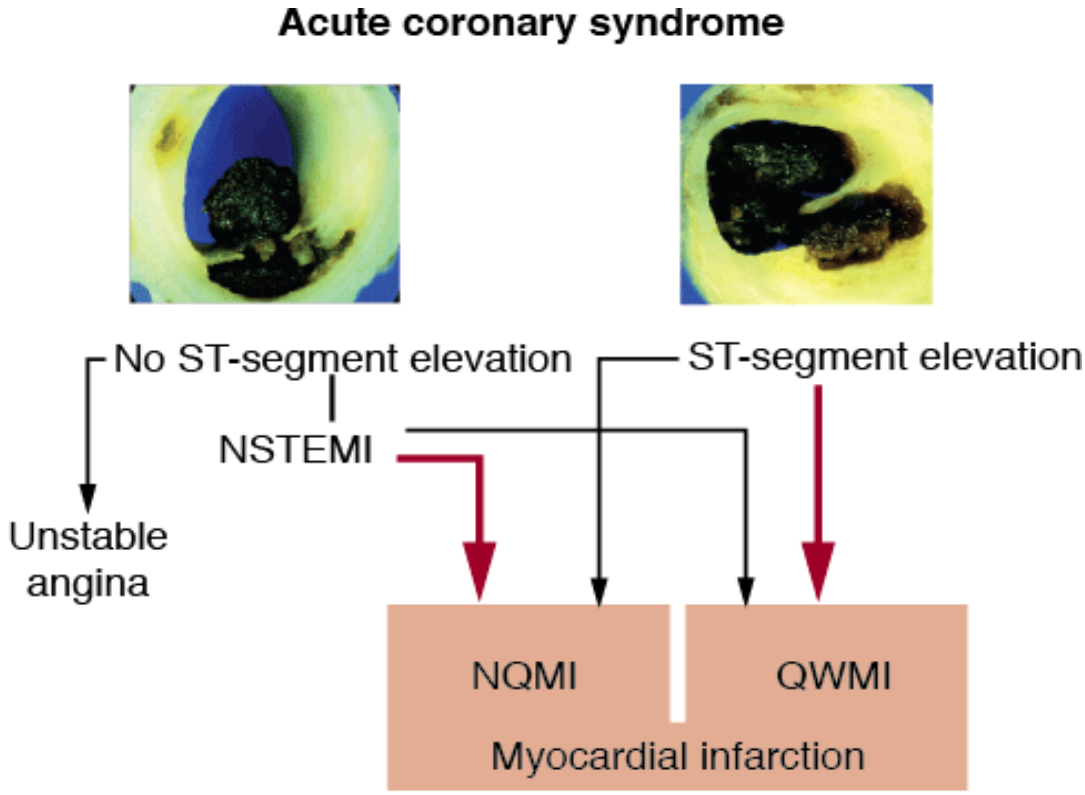


Fig.3: Acute Coronary Syndrome

NQMI- Non Q-Wave myocardial infarction.

QWMI- Q-Wave Myocardial Infarction.

MI is classified into five main types:

Type 1 – Spontaneous MI related to ischemia due to a primary coronary event such as plaque erosion and/or rupture, fissuring, or dissection.

Type 2 – MI secondary to ischemia due to either increased oxygen demand or decreased supply, e.g. coronary artery spasm, coronary embolism, anemia, arrhythmias, hypertension, or hypotension

Type 3 – Sudden unexpected cardiac death, including cardiac arrest, often with Symptoms suggestive of myocardial ischemia, accompanied

by new ST Elevation, or new left bundle branch block (LBBB),

Type 4 – Associated with coronary angioplasty or stents.

- Type 4a – MI associated with percutaneous coronary intervention (PCI).
- Type 4b – MI associated with stent thrombosis as documented by angiography or at autopsy.

Type 5 – MI associated with CABG.

The terms Q wave and non-Q wave MI were previously used to indicate STEMI and non-STEMI respectively [17].

2.1.4. ETIOLOGY

Acute coronary syndromes (ACS) are associated with structurally as well as functionally complex plaques and coronary artery stenoses, coronary endothelial lesions, and plaque inflammation[18]. Structural morphology, cellular composition, and biological activity of coronary plaques appear to be closely linked. Plaque instability correlated more with biological activity and cellular composition than with angiographical findings[19]. Exogenous factors (e.g. mechanical stress, vasomotor tone, infection, blood viscosity, coagulability) further modify such interaction, making the final outcome even less predictable. Systemic or multi-focal arterial inflammation may be independent risk factors for acute coronary events.

Thrombosis – The most common cause

The common cause of an MI is a blood clot (thrombosis) that forms inside a coronary artery or one of its branches and blocks the blood flow to a part of the heart.

Blood clots do not usually form in normal arteries. However, a clot may form if there is some atheroma within the lining of the artery. Atheroma is the technical term for fatty patches or 'plaques'. Plaques of atheroma may gradually form over a number of years in one or more places in the coronary arteries. Each plaque has an outer firm shell with a soft inner fatty core. What happens is that a 'crack' (plaque rupture) develops in the outer shell of the atheroma plaque. This exposes the softer inner core of the plaque to blood and can trigger the clotting mechanism in the blood to form a blood clot. Therefore, a buildup of atheroma is the root problem that leads to most cases of MI [20].

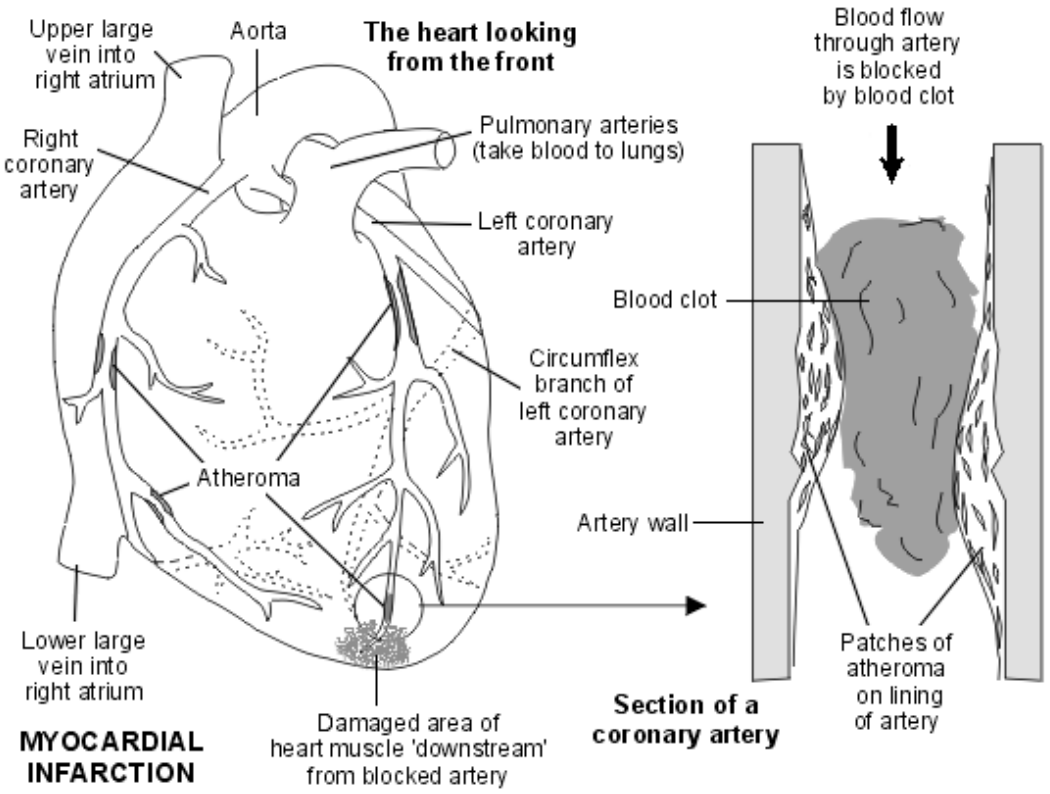


Fig .4: Atheroma formation in Heart

2.1.5. Complication of myocardial infarction

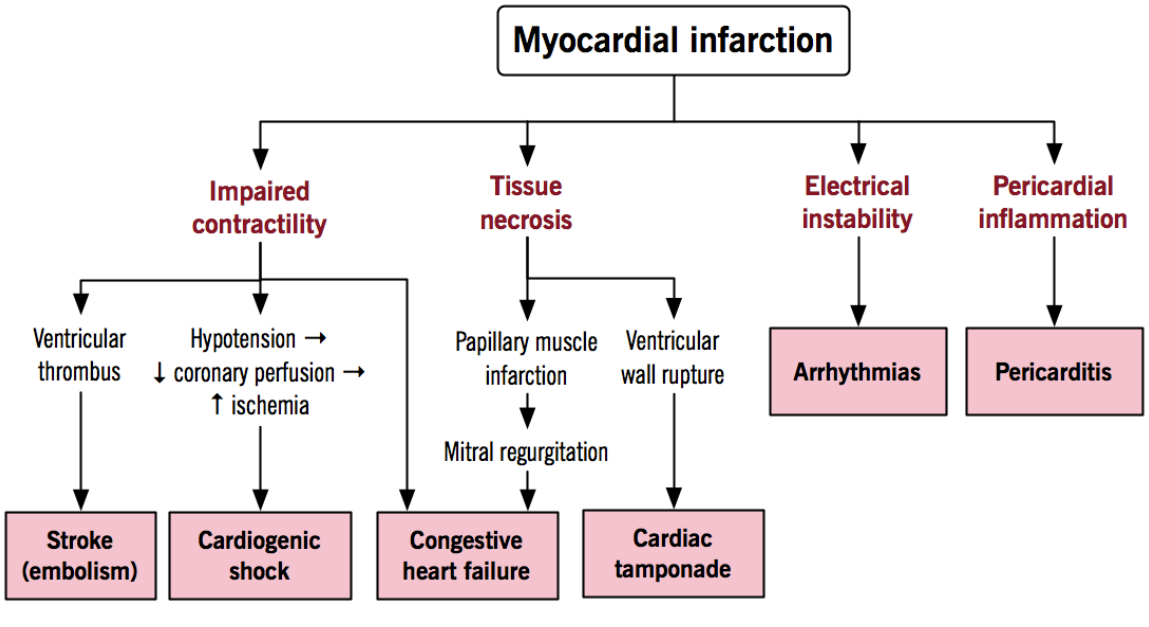


Fig.5 : Complication of myocardial infarction

Complications of MI are failure of reperfusion (ischemic), cardiac rupture, thrombosis and emboli, heart failure, psychological complications including depression and pericarditis, contractile dysfunction, arrhythmias, papillary muscle dysfunction[21].The pumping ability of heart is reduced, if a large area of heart muscle is damaged. This causes the occurrence of other complications after myocardial infarction because less blood is pumped around body. These complications include heart failure, swollen ankles, tiredness and breathlessness. If electrical activity of heart is affected, abnormal heart rhythms, fast or chaotic heart beats may occur. An immediate electrical shock treatment given by defibrillator is needed. If further buildup of atheroma continues or coronary arteries are badly affected, there is more likely to have the occurrence of myocardial infarction in future[22].

MAJOR COMPLICATION IS HEART FAILURE

Heart failure (HF) is a frequent complication of myocardial infarction (MI). Several factors as follows,

- Myocardial ischemia
- Infarct size
- Ventricular remodeling
- Stunned myocardium
- Mechanical complications, and hibernating
- Myocardium influence the appearance of left ventricular systolic dysfunction with or without clinical HF after MI [23].

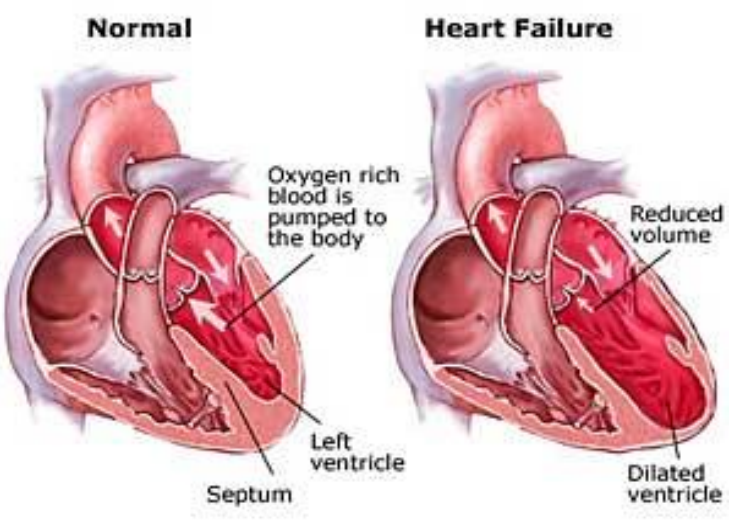


Fig .6: Structure of normal and abnormal heart

2.1.6. RISK FACTORS FOR MYOCARDIAL INFARCTION

Predisposing risk factors for myocardial infarction are generally divided into two categories.

- Non -modifiable risk factors
- Modifiable risk factors

According to Interheart study, risk factors for MI are divided into 2 categories i.e.

- Emerging risk factors (homocysteine, glucose abnormalities, nutritional factors, abdominal obesity and psychosocial factors) and
- Conventional risk factors (hypertension, diabetes, smoking and elevated cholesterol) between people of varying geographic and ethnic origin. However, these known risk factors would explain only about 50% of cases of heart disease. Moderate or strenuous exercise, consumption of alcohol (≥ 3 times per week) and daily consumption of

fruits and vegetables prove to be protective[24]. Nine different factors predispose the risks of MI worldwide.

2.1.6.1.MODIFIABLE PREDISPOSING RISK FACTORS

- **Smoking**

Smoking is considered as strong risk factor for myocardial infarction, premature atherosclerosis and sudden cardiac death. Smoking results in early STEMI especially in otherwise healthier patients. Smoking causes an average of 7 years earlier and more likely twice the chances of infarction than non smokers[30].

- **Physical activity**

Inactive people with multiple cardiac risk factors are more likely to develop MI. To get benefit, these individuals should start from modest exercise training. There should be aggressive risk factor modification before performance of vigorous activity.

- **LDL and triglyceride levels**

Elevated triglyceride levels and dense, small LDL particles act as predisposing risk factors for MI. Non fasting triglyceride levels appear to be a strong and independent predictor of future risk of MI, particularly when the total cholesterol level is also elevated. The reason behind it is that decreased HDL-C levels and increased triglyceride levels cause metabolic perturbations thus causing adverse consequences. To identify high risk individuals, elevated triglyceride levels may become markers.

- **Obesity/ Body mass index (BMI)**

Increased BMI is directly related to incidence of MI. Infarction is greatly enhanced by extreme obesity because it is a recognized risk factor for MI. To reduce the population burden of MI in US, strategies are devised to promote optimal body weight[25].

- **Diabetes mellitus (DM)**

Significant differences in parameters measured were noted when all diabetic and non-diabetic patients were compared to the control group. It was found that in men with myocardial infarction there are significant differences between diabetic and nondiabetic patients with respect to certain risk factors such as age, hypertension and hypertriglyceridemia in diabetic patients, while smoking and family history are predominant factors in non diabetic patients. However, newly diagnosed diabetic men have similar risk profiles to their known diabetic counterparts.

- **Hypertension**

Hypertension is strong and independent risk factor for MI. It is major risk factor of causing atherosclerosis in coronary blood vessels, result in heart attack or MI. Hypertension and MI are closely linked.

- **Psychosocial Stress**

Chronic life stress, social isolation and anxiety increase the risk of heart attack and stroke.

2.1.6.2. NON -MODIFIABLE PREDISPOSING RISK FACTORS

Increasing age is more likely to die of heart disease. About 80% of heart disease deaths occur in people aged 65 or older. Gender Men tend to have heart attacks earlier in life than women. Women's rate of heart attack increases after menopause, but does not equal men's rate. Even so, heart disease is the leading cause of death for both men and women.

- **Heredity/Family history**

Increased risk, if a first degree blood relative had coronary heart disease or stroke before the age of 55 years for male relative and 65 years for female relatives.

- **Genetic factor**

Coronary artery disease and myocardial infarction are the most frequent causes of death. Even nowadays, every second myocardial infarction is lethal and hits the patients unexpectedly without previous signs or symptoms. These are the risk factors which causes MI.

2.2. NANOPARTICLES

Nano particles are particles between 1 and 100 nanometer (nm) in size with a surrounding interfacial layer. The interfacial layer is an integral part of nanoscale matter, fundamentally affecting all of its properties. The interfacial layer typically consists of ions, inorganic and organic molecules. Organic molecules coating inorganic nanoparticles are known as stabilizers, capping and surface ligands, or passivating agents[26]. In nanotechnology a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Particles are further classified according to diameter[27].

2.2.1. Classification of Nanoparticles

Nanoparticles are broadly classified in to three classifIcations[28].

• **One dimension nanoparticles**

One dimensional system (thin film or manufactured surfaces) has been used fordecades. Thin films (sizes 1–100 nm) or monolayer is now common place in the field of solar cells offering, different technological applications, such as chemical and biological sensors, information storage systems, magneto-optic and optical device, fiber-optic systems.

• **Two dimension nanoparticle**

Carbon nanotubes.

• **Three dimension nanoparticles**

Dendrimers, Quantum Dots, Fullerenes (Carbon 60), (QDs)

2.2.2. Characterization of Nanoparticles

Characterization of nanoparticles is based on the size, morphology and surface Charge, using such advanced microscopic techniques as atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Properties such as the size distribution, average particle diameter, and charge affect the physical stability and the in vivo distribution of the nanoparticles. Properties like surface morphology; size and overall shape are determined by electron microscopy techniques. Features like physical stability and redispersibility of the polymer dispersion as well as their *in vivo* performance are affected by the surface charge of the nanoparticles. It is very important to evaluate the surface charge during characterization of nanoparticles.

2.2.2.1. Particle Size

Characterizations of nanoparticles are primarily evaluated by the particle size distribution and morphology. With the aid of electron microscopy it's now possible to ascertain the morphology as well as the size of nanoparticles. Application of nanoparticles in drug release and drug targeting can be conveniently determined by various tools. It has already been reported that particle size of nanoparticles has profound effect on the drug release.

Smaller the size of nanoparticles larger surface area, which results in to fast drug release. Loaded drug when exposed to the particle surface area causes significant drug release. In contrast, inside the nanoparticles drugs slow diffusion of larger particles occurs.

Consequently smaller particles tend to aggregate during storage and transportation of nanoparticle dispersion. Therefore there is a mutual compromise between maximum stability and small size of nanoparticles[29]. In addition degradation

of the polymer can also be affected by the particle size e.g. the extent of poly (lactic-co-glycolic acid) degradation was found to increase with increasing particle size in vitro[30].

2.2.2.2.B. Scanning Electron Microscopy (SEM)

This electron microscopy based technique determines the size, shape and surface morphology with direct visualization of the nanoparticles. Therefore scanning electron microscopy offer several advantages in morphological and sizing analysis. However they provide limited information about the size distribution and true population average.

During the process of SEM characterization, solution of nanoparticles should be initially converted into a dry powder. This dry powder is then further mounted on a sample holder followed by coating with a conductive metal (e.g. gold) using a sputter coater. Whole sample is then analyzed by scanning with a focused fine beam of electrons[31].

Secondary electrons emitted from the sample surface determine the surface characteristics of the sample. This electron beam can often damage the polymer of the nanoparticles which must be able to withstand vacuum. Average mean size evaluated by SEM is comparable with results obtained by dynamic light scattering. In addition these techniques are time consuming, costly and frequently need complementary information about sizing distribution.

2.2.2.4. Transmission Electron Microscope

Transmission electron microscopy techniques can provide imaging, diffraction and spectroscopic information, either simultaneously or in a serial manner, of the specimen with an atomic or a sub-nanometre spatial resolution.

TEM operates on different principle than SEM, yet it often brings same type of data. The sample preparation for TEM is complex and time consuming because of its requirement to be ultra thin for the electron transmittance[32].High-resolution TEM imaging, when combined with nano diffraction, atomic resolution electron energy-loss spectroscopy and nanometre resolution X-ray energy dispersive spectroscopy techniques, is critical to the fundamental studies of importance to nanoscience and nanotechnology.

During the TEM characterization nanoparticles dispersion is deposited onto support grids or films[33]. After dispersion they are fixed using either a negative staining material (phosphotungstic acid or derivatives, uranyl acetate, etc., or by plastic embedding).

2.2.2.5. Surface Charge

Surface charge and intensity determines the interaction of nanoparticles with the biological environment as well as their electrostatic interaction with bioactive compounds. Stability of colloidal material is usually analyzed through zeta potential of nanoparticles.

Zeta potential is an indirect measure of the surface charge. It can be obtained by evaluating the potential difference between the outer Helmholtz plane and the surface of shear. Thus zeta potential of colloidal based dispersion assists indirectly evaluating its storage stability. Zeta potential values (high zeta potential

values, either positive or negative) are achieved in order to ensure stability and avoid aggregation of the particles.

Zeta potential values can be utilized in evaluating surface hydrophobicity and the nature of material encapsulated within the nanocapsules
or coated onto the surface[34].

2.2.2.6. Surface Hydrophobicity

Techniques such as hydrophobic interaction chromatography, biphasic partitioning, adsorption of probes, contact angle measurements etc. can be utilized for the determination of surface hydrophobicity. Recent advancement in research offers several sophisticated analytical tools for surface property analysis of nanoparticles. Modern technique such as X-ray photon correlation spectroscopy not only determine surface hydrophobicity but also permits the identification of specific chemical groups on the surface of nanoparticles[35].

2.2.2.7. Drug Release

It's very essential to determine extent of the drug release and in order to obtain such information most release methods require that the drug and its delivery vehicle be separated. Drug loading capacity of the nanoparticles is defined as the amount of drug bound per mass of polymer or in another term it is the moles of drug per mg polymer or mg drug per mg polymer or it could also be given as percentage relative to the polymer[36.]

Various techniques such as UV spectroscopy or high performance liquid chromatography (HPLC) after ultracentrifugation, ultra filtration, gel filtration, or centrifugal ultra filtration are used to determine this parameter. Methods that are

employed for drug release analysis are also similar to drug loading assay which is more often assessed for a period of time to evaluate the drug release mechanism.

2.2.3. Preparation of Nanoparticles

The preparation of nanoparticles depends on the physicochemical character of the polymer and the drug to be loaded. Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including[37].

- Antigencity of the final product.
- Biocompatibility and toxicity
- Degree of biodegradability
- Drug release profile desired
- Inherent properties of the drug (aqueous solubility and stability)
- Size of nanoparticles required
- Surface characteristics (charge and permeability)

Nanoparticles have been usually prepared by three methods:

- Dispersion of preformed polymers
- Ionic gelatin or coacervation of hydrophilic polymers
- Polymerization of monomers

2.2.4. Properties of nanoparticles

- ✓ Nanoparticles are of great interest because of their unique properties.

- ✓ Bulk material has constant physical properties regardless of its size, the properties of materials change as their size reduces to nanoscale.
- ✓ Nanoparticles have large surface area to volume ratio as compared to its bulk material.
- ✓ Nanoparticles are very small in size due to which quantum effects arise and hence nanoparticles have optical properties.
- ✓ Clay nanoparticles are integrated into polymer matrices to increases the reinforcement leading to stronger plastics.
- ✓ Nanoparticles are semiconductor devices, radiation therapy, solar cells, synthetic fibers etc
- ✓ Nanoparticles can be used for anti-reflection product coatings.

2.2.5. Green synthesis of nanoparticles

"Green synthesis" of nanoparticles makes use of environmental friendly, non-toxic and safe reagents. Nano particles synthesized using biological techniques or green technology have diverse natures, with greater stability and appropriate dimensions since they are synthesized using a one-step procedure[38].

Nanoparticles can be synthesized using a variety of methods including chemical, physical, biological, and hybrid techniques.

Physical methods including plasma arcing, ball milling, laser desorption, lithographic techniques, sputter deposition, layer by layer growth, molecular beam epitasis and diffusion flame synthesis of nanoparticles. Similarly, chemical methods are used to synthesize NPs by electro deposition, sol–gel process, chemical solution

deposition, chemical vapour deposition soft chemical method, Catalytic route, hydrolysis co-precipitation method and wet chemical method [39].

Chemical and Physical methods have been using high radiation and highly concentrated reductants and stabilizing agents that are harmful for the environmental and to human health. Hence, biological synthesis of nanoparticles is a single step bio-reduction method and less energy is used to synthesize eco-friendly.

Advantages of green synthesis

- ✓ Green synthesis encapsulate the nanoparticles. So prevent instant reaction.
- ✓ Easily scaled up for large synthesis of nanoparticles
- ✓ No need of high temperature, pressure, energy, and toxic chemicals
- ✓ Maintenance cost is much less
- ✓ Acts as both reducing and stabilising agents.
- ✓ Reduce toxicity
- ✓ Highly stable and rapid synthesis
- ✓ Environmental friendly

2.2.6. Metal ions used in nanoparticles

- ✓ Gold
- ✓ Silver
- ✓ Zinc
- ✓ Iron
- ✓ Titanium

2.2.7. Gold nanoparticles

A gold-based nanoparticle has reported to be effective of antibacterial agents. Transcriptomic and proteomic data show the down-regulation of atpD and atpA, which are subunits of F-type ATP synthase. The down-regulation could lead to a decrease of the activity of F-type ATP synthase. To test this hypothesis, we extracted the membrane protein of gold NP-treated or untreated E. coli and determined the activity of F-type ATP synthase.

Gold NPs can severely decrease the activity of F-type ATP synthase F-type ATP synthase plays a major role in the process of ATP synthesis; its decrease can directly lead to the decrease of the ATP level. As expected, we found that the ATP level significantly decreased in NP-treated E. coli. The biological function of ATP synthase is dependent on the membrane potential. We further investigated the effects of gold NPs on cytoplasmic membrane potential of E. coli via a fluorescent probe, DiSC3 (5) dye, which can be quenched by the electrically polarized membrane[40].

When the membrane potential collapses, the probe releases into the cytoplasm and leads to an increase in fluorescence. The fluorescence of NP-treated E. coli was significantly increased compared with that of untreated E. Coli.

- Gold NPs induce the down-regulation of oxidative phosphorylation pathway (Ftype ATP synthase and ATP level) and ribosome pathways, and the transient upregulation of chemotaxis. Gold NPs do not induce the change of ROS-related processes.

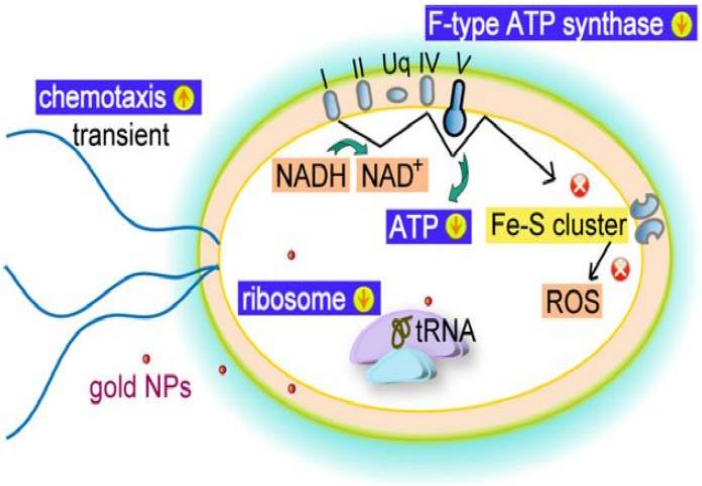


Fig.No :7 Mechanism of action of gold nanoparticles in bactericidal agents

2.2.8. Silver nanoparticles

Silver nanoparticles are nanoparticles of silver which are in the range of 1 and 100 nm in size. Silver nanoparticles have unique properties which help in molecular diagnostics, in therapies, as well as in devices that are used in several medical procedures.

The major methods used for silver nanoparticle synthesis are the physical and chemical methods[41]. The major biological systems involved in this are bacteria, fungi, and plant extracts. The major applications of silver nanoparticles in the medical field include diagnostic applications and therapeutic applications. In most of the therapeutic applications, it is majorly used antimicrobial property.

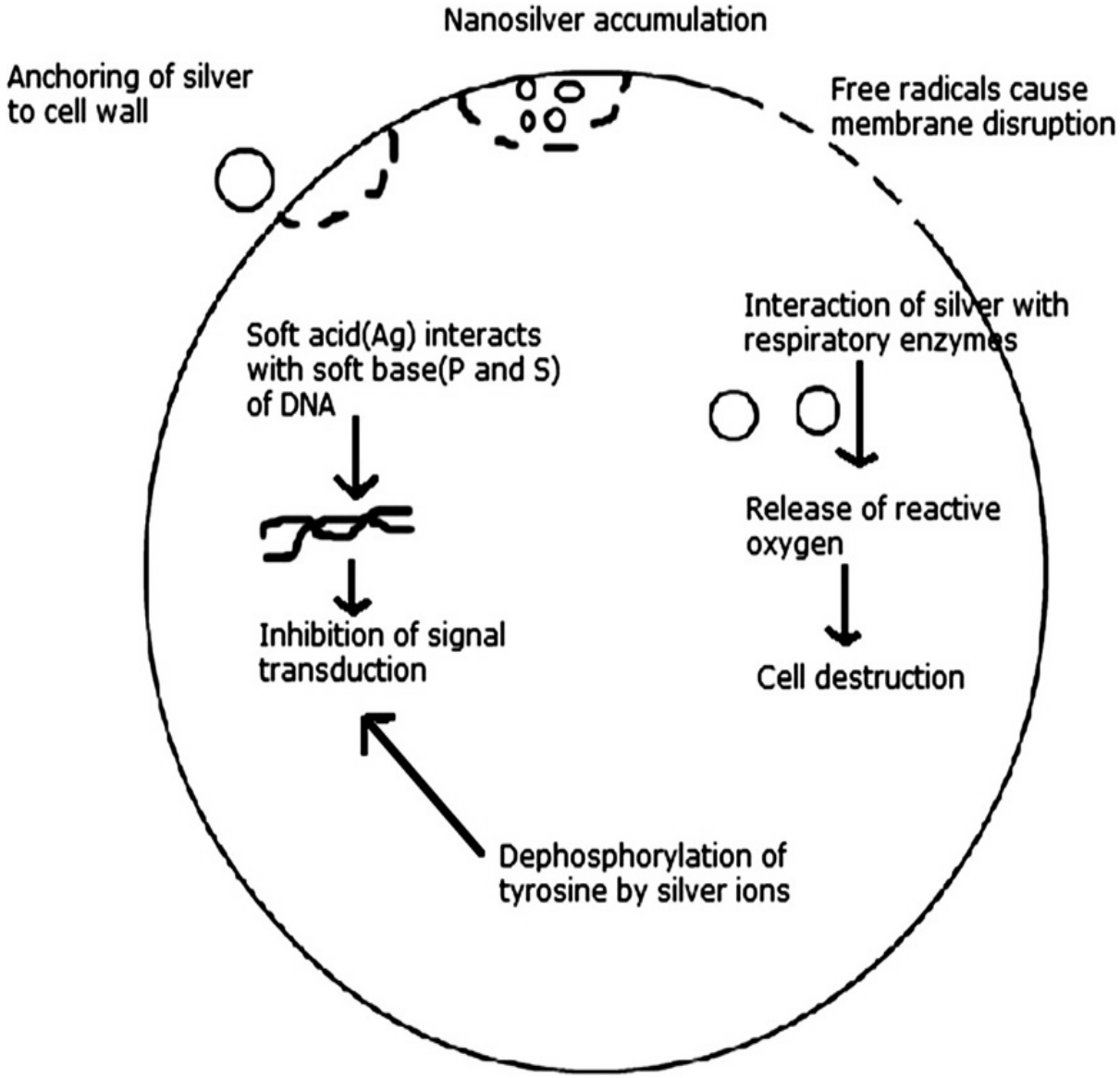


Fig.No: 8 Mechanism of action of silver nanoparticles in bacteria

2.2.9. Zinc nanopartiocles

Zinc is an essential metallic micronutrient with a potential association with CVD. The importance of zinc is apparent from the enormous number of proteins that

contain zinc ions in their structure[41]. Intracellular zinc plays a critical role in the redox signaling pathway, where by deficiency of zinc under oxidative stress leads to the degradation of critical proteins, as shown for protein kinase C (PKC) .

Zinc plays a role in cardiovascular physiology and pathology. Several investigators have shown decreased blood zinc levels in Patients with ischemia/myocardial infarction, congestive heart failure, conduction abnormalities, and heart transplant, resulting in poor outcomes.

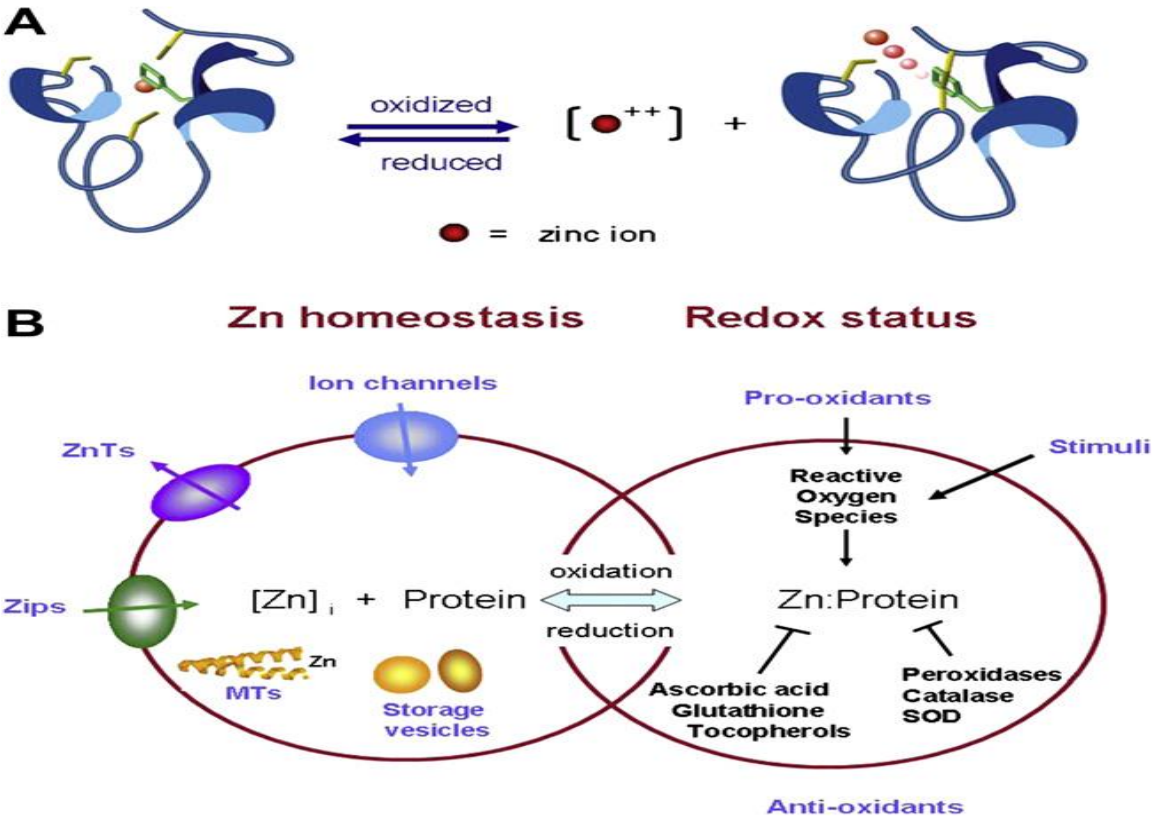


Fig.No:9 Intracellular role of zinc homeostasis

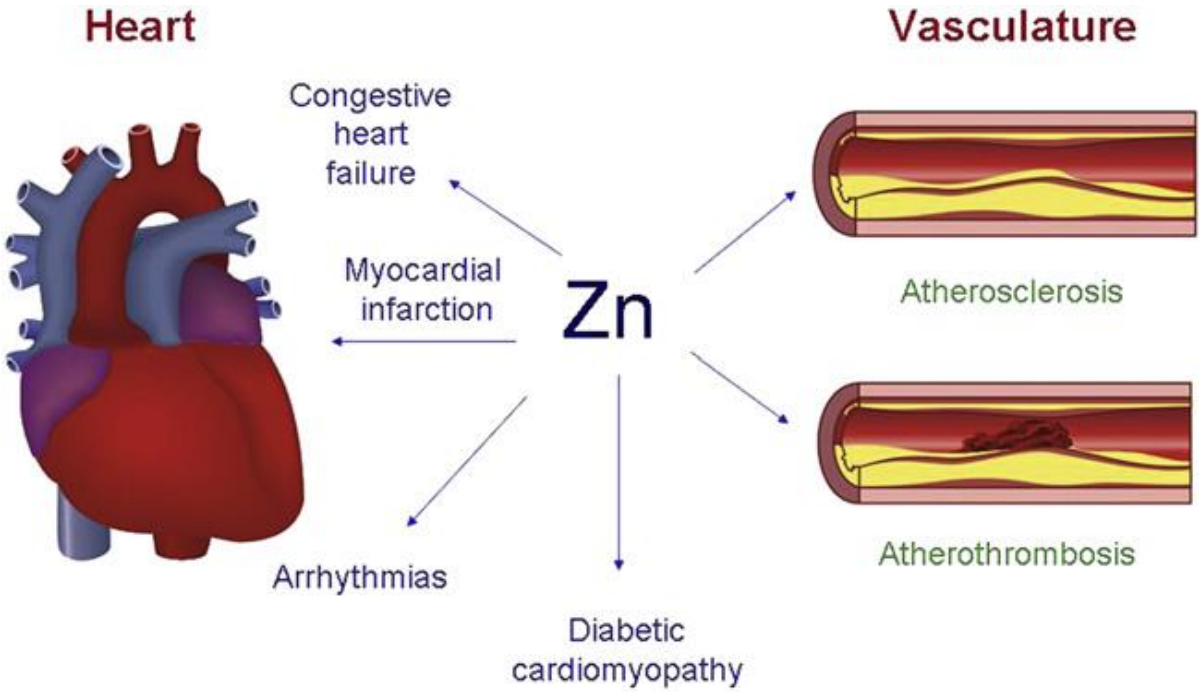


Fig.No: 10 Role of zinc ions in cardiovascular system

2.2.10. Iron nanopartiocles

Iron nanoparticles have been employed for targeted delivery systems for the delivery of DNA enzyme for the treatment of hepatitis C. The nanoparticles induced the knock down of hepatitis C virus gene, NS3. HCV NS3 gene encodes helicase and proteases which are useful for viral replication[42].

The nano formulation did not suffer from severe immune responses. In vivo evaluation on mice showed that after administration on the animal models, the nanoparticles accumulated in the hepatocytes and macrophages in the liver suggesting their potential application for the treatment of hepatitis C.

2.2.11. Titanium nanoparticles

Because of its photosensitivity, nano-TiO2 can become a substance that generates reactive oxygen species (ROS) in the body[43]. Under normal conditions, ROS play an important role in anti-bacterial and anti-inflammatory processes and can suppress tumours. However, disease or some exogenous poisons may cause disorders in the body's anti-oxidation system, resulting in radical metabolic imbalances and abnormal increases in ROS. Excessive ROS produce toxicity, resulting in the formation of biofilms and macromolecular substances that induce lipid peroxidation damage.

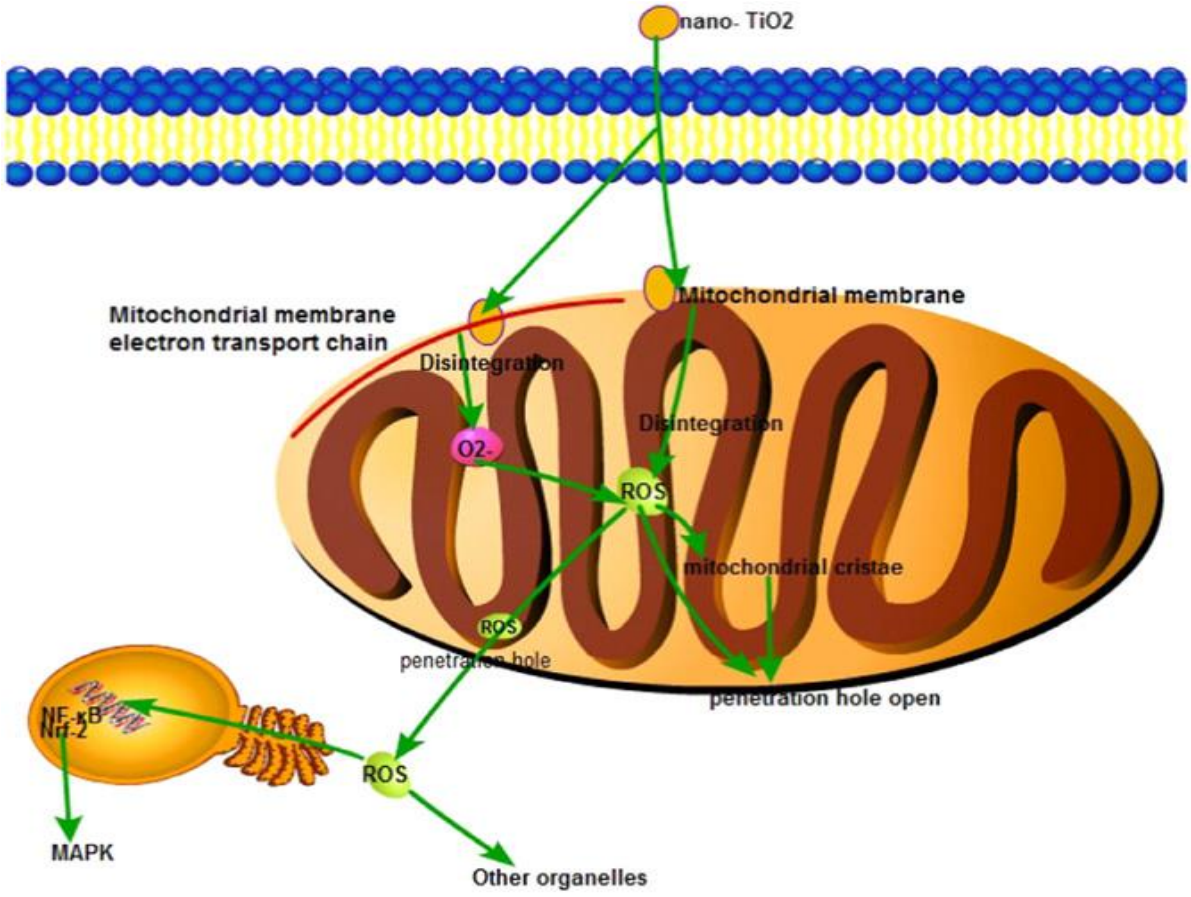


Fig.No:11 Process by which TiO2 nanoparticles produce ROS in mitochondria.

2.3. *NYCTANTHUS ARBOR-TRISTIS*

Nyctanthus arbor-tristis Linn is a small scared ornamental tree known across the country for its fragrant while flower[44]. *Nyctanthus arbor-tristis* Linn is commonly known as Night jasmine or parijata.

Plant name : *Nyctanthus arbor-tristis*

Family : Oleaceae

2.3.1. Vernacular names

Tamil	:	Pavala- malligai, Manjhapu
English	:	Night jasmine, Coral jasmine
Hindi	:	Harsinghar, Seoli, Sihau
Sanskrit	:	Parijatha, parijatah, sephalika
Malayalam	:	Mnnapu, Pavizhamalli, Parijatak
Telugu	:	Kapilanagadustu, Pagadamalle
Kannada	:	Goli, Harsing, Parijata
Marathi	:	Kharbadi, Kharassi, Khurasli.
Gujarathi	:	Jayaparvati
Punjabi	:	Harsinghar

2.3.2. Taxonomical name

Kingdom : Planate

Order : Lamiale

Division : Magnoliophyt

Class : Magnoliopsida

Family : Oleaceae

Genus : Nyctanthes

Species : Arbortristis[45].

2.3.3. Plant diagram

Fig.No:12 Aerial parts of *Nyctanthus arbor-tristis* & Powder of *Nyctanthus arbor-tristis*

2.3.4. Morphology

Colour: Light to dark green

Odour: Indistinct

Taste: Bitter and astringent

Leaf: Simple, 5-14 cm long, 2.5-7.5 cm wide, ovate, acute

Margin: Entire or distinctly toothed Base and Round

Venation: Reticulate, lateral vein 3-6 pairs

2.3.5. Phytoconstituents of *Nyctanthus arbor- tristis*

2.3.5.1. Leaves

Three new benzoic esters of Loganin and 6-β-hydroxy loganin, namely Arborside-A, Arborside-B, and Arborside-C were found to be present in the leaves. From leaves 10-Benzoylnyctanthoside named as Arborside-D were isolated. Other iridoid glycosides that were reported are 6, 7-Di-O-benzoyl nyctanthoside, 6-O-transcinnamoyl-6-β-hydroxy loganin and 7-O-trans cinnamoyl-6-β-hydroxy loganin from the leaves. A phenyl propanoid glucoside Des rhamnosyl verbascoside was reported from the leaves. Leaves also contain the alkaloid Nyctanthine along with Mannitol, β-Amyrin β-Sitosterol, Hentriacontane, Benzoic acid, Astragalin, Nicotiflorin, Oleanolic acid, Nyctanthic acid, Friedelin and Lupeol[46].

2.3.5.2. Flowers

Essential oil, nyctanthin, d- mannitol, tannin and glucose, carotenoid, Glycosides, β- mono gentiobioside, β-D monoglucoside ester of α-crocetin, β-digentiobioside ester of α-crocetin.

2.3.5.3. Stem

Glycoside-naringenin-4'-o**-** β-glucapyranosyl- α-xylopyranoside was screened from the stem chromatographed the chloroform extract of the stem over silica gel column and

reported the presence of β-Amyrin, Arbortristoside-A, Oleanolic acid, Nyctoside-A, Nyctantic acid and 6-β-hydroxyloganin.

2.3.5.4. Seeds

Seeds give a water soluble polysaccharide containing D-Glucose and D-Mannose, indicating that the polysaccharide is a glucomannan. Iridoid glycosides Arbortristoside-A, Arbortristoside B, Arbortristoside-C and 6-β-hydroxyloganin have been isolated. Further examination of the seeds led to the isolation and identification of two minor iridoid glucosides, Arbortristoside-D and Arbortristoside-E together with the previously reported Arbortristoside-B. Other iridoid glucoside reported are Nyctanthoside, A phenyl propanoid glucoside, Nyctoside-A was isolated from the methanolic extract of the seeds.

2.3.6 Traditional uses

2.3.6.1 Leaves

The leaves of *Nyctanthus arbor-tristis* Linn. are mainly used in ayurvedic. The Parijatha is regarded in Hindu mythology as one of the five grating trees of Devaloka[47].

Different parts of Nyctanthus arbor-tristis Linn are used in Ayurveda, Sidda, and Unani system of medicines. Treatment of various diseases such as Sciatica, chronic fever, rheumatism,and internal worm infections, and as laxative, diaphoretic and diuretic[48].Leaves are mainly used in the enlargement of spleen. Paste of leaves is mixed with honey for the treatment of blood pressure, diabetes and fever[49].The decoction of the leaves is mainly used by Ayurvedic physicians for the treatment of arthritis, malaria, intestinal worms, obstinate sciatica, cholagogue and laxative. The leaf juice is used to treat loss of appetite, piles, liver disorders, biliary disorders, intestinal worms, chronic fever, obstinate sciatica, rheumatism and fever with rigors. The extracted juice of leaves

acts as a cholagogue, laxative and mild bitter tonic. It is given with little sugar to children as a remedy for intestinal ailments [50].

2.3.6.2. Flowers

The flowers are used as stomachic, carminative, astringent to bowel, antibilious, expectorant, hair tonic and in the treatment of piles and various skin diseases and in the treatment of ophthalmic purposes. The bright orange corolla tubes of the flowers contain a colouring substance nyctanthin, which is identical with Crocetin from Saffron. The corolla tubes were formerly used for dyeing silk, sometimes together with Safflower or turmeric[51].

2.3.6.3. Stem

e powdered stem bark is given in rheumatic joint pain. Used as expectorant. Bark is ıinly used for the snakebite and bronchitis[52].

2.3.6.4. Seed

The seeds are used as anthelmintics and in alopecia. It is antibilious and an pectorant, and is also useful in bilious fever. The powdered seeds are used to cure scurfy ections of scalp, piles and skin diseases [53].

2.3.7. Pharmacological action of *N. arbor- tristis*

- Antibacterial activity
- Anticancer activity
- Anthelmintic activity
- Analgesic and Anti inflammatory activity
- Hypoglycemic activity
- Larvicidal activity

- Invitro anti-oxidant activity
- Immuno pharmacological activity

2.3.7.1 Antibacterial activity

The antibacterial activities of *Nyctanthus arbor- tristis* L., seed and fruit extract were used for their antibacterial screening. Melanin content and stability of the fruit and seed were studied against various factors like temperature, oxidants and metal ions. Phytochemical analysis shows the presence of phytosterols. Steroids were present in seeds. UV spectral studies show high content of melanin in seeds[54]. Chloroform and ethyl acetate extracts of fresh leaf, seeds and fruits were showed significant antibacterial activity against Gram negative bacteria (E.coli and K. Pneumonise) and Gram positive bacteria (S.aureus), where as dried extract of chloroform and ethyl acetate shown significant antibacterial activity against pseudomonas aeruginosa.

2.3.7.2 Anticancer activity

Methanol extract of fruit, leaf and stem of *N.arbor-tristis* were tested in vitro cancer activities. Moderate activity was observed at 30mg/ml conc. with 71% inhibition of dried NAT leaf methanol extract and least inhibitory activity was observed at 10mg/ml conc. With 86% inhibition of Breast cancer cell lines free of pathogens. The phytochemicals isolated from NAT dried fruits methanol are glycosides, tannins, phenols and steroids and are predicted to responsible for this cancer activities[55].

2.3.7.3 Anthelmintic activity

In vitro anthelmintic activity of nyctanthus arbor-tristis Linn bark, parasite diseases causes severe morbidity by affect the population. More than half of the population of the world suffers various types of infections and suffer from worm

infections. Alcohol and aqueous extracts from the bark of *Nyctanthus arbor- tristis* Linn were investigated for their anthelmintic activity against pheretima posthuma. Three concentrations (20,40and 60 mg/ml) of each extracts were studied. It involves the determination of time of paralysis and time of death of the worm. Albendazole in same concentration as that of extract was included as standard reference and distilled water as control. The anthelmintic activity of alcohol and aqueous extracts of *Nyctanthes arbor-tristis* Linn has therefore been compared and demonstrated for the first time [56].

2.3.7.4 Analgesic and Anti inflammatory activity

The analgesic activity of aqueous and ethanolic leaves extract of *Nyctanthes arbor-tristis,* it was found from the percentage inhibition index that ethanolic extract shown better analgesic than aqueous extract when compared with standard drug aspirin. The methanolic extract of the stem bark of *Nyctanthes arbor-tristis* shows statistically significant analgesic activity (by all four applied models) compared with control, standard. The result of treatment with the extracts of *Nyctanthes arbor-tristis* was similar with the standard and it showed significant analgesic activity[57]. Petroleum ether extract was found to be most active for analgesic activity and hence subjected to activity-guided fractionation.

The significant and dose-dependent activity showed by β-sitosterol (5, 10 and 20 mg/kg, i.p.) comparable with the standard extract. β-Sitosterol from *N. arbor-tristis* leaves might be responsible for analgesic and anti-inflammatory activity[58]. The ethanolic extract obtained from the orange tubular of calyx of NAT and the isolated carotenoid (200 mg/kg, i.p.) possess significant inhibition of carangeenan-induced rat paw oedema using diclofenac sodium as a standard drug. The anti-inflammatory

activity against acute inflammatory oedema in rats using different phlogiston agents like carrageen in, formalin, histamine, 5-hyroxytrypatamine and hyluronidase significantly showed by aqueous soluble fractions of NAT ethanolic extract[59].

2.3.7.5. Hypoglycemic activity

Hypoglycemic activity of methanolic extracts of *Nyctanthes arbor-tristis* Linn. root in alloxan induced diabetic rats; This research aims to investigate the hypoglycemic activity of methanolic extract of *Nyctanthes arbor -tristis* Linn. Root in alloxan induced diabetic albino rats. A comparison was made between the action of *Nyctanthes arbor-tristis* Linn. methanolic extract and a known anti diabetic drug glibenclamide (0.5mg/kg p.o).The methanolic extract of Nyctanthes arbor-tristis Linn. root was administered orally at different doses to normal rats. The methanol extract at 500 mg/kg dose level exhibited significant ($p<0.05$) hypoglycemic activity[60]. The ant diabetic activity of methanol extract of root of *Nyctanthes arbor-tristis Linn* is comparable to that of diabetic control animals. It is concluded that methanol extract of root of Nyctanthes arbor-tristis Linn possess safe and strong anti diabetic activity.

2.3.7.6. Larvicidal activity

The larvicidal activity of saraca indica, *Nyctanthes arbor-tritis*, and clitoria ternatea extracts against three against three mosquito vector species, Screening of natural products for mosquito larvicidal activity against three major mosquito vectors Aedes aegypti, Culex quinquefasciatus, and Anopheles stephensi resulted in the identification of three potential plant extracts viz., Saraca indica/asoca, *Nyctanthes arbor-tristis* L., and clitoria ternate for mosquito larval control. In the case of S. indica/asoca, the petroleum ether extract of the leaves and the chloroform extract of the bark were effective against the larvae of C. quinquefasciatus with respective LC

(50) values 228.9 and 291.5 ppm. The LC (50) values of chloroform extract of N. arbor-tristis leaves were 303.2, 518.2, and 420.2 ppm against A. aegypti, A. stephensi, and C. quinquefasciatus, respectively. The methanol and chloroform extracts of flowers of *N. arbor-tristis* showed larvicidal activity against larvae of A. stephensi with the respective LC (50) values of 244.4 and 747.7 ppm. Among the methanol extracts of C. ternatea leaves, roots, flowers, and seeds, the seed extract was effective against the larvae of all the three species with LC(50) values 65.2, 154.5, and 54.4 ppm, respectively, for A. stephensi, A. aegypti, and C. quinquefasciatus. Among the three plant species studied for mosquito larvicidal activity, C. ternatea produced the most promising mosquito larvicidal activity. The phytochemical analysis of the promising methanolic extract of the seed extract was positive for carbohydrates, saponins, terpenoids, tannins, and proteins. In conclusion, bioassay-guided fractionation of effective extracts may result in identification of a useful molecule for the control of mosquito vectors[61].

2.3.7.7. In vitro anti-oxidant activity

Under most pathological conditions there is generation of reactive oxygen species and other free radicals. An increase in the antioxidant reserves of the organism can reduce oxidative stress and some of the plant-derived agents may help to reduce it. *Nyctanthes arbor-tristis* leaf extracts are extensively used in Indian traditional Medicine. In the present study we have examined the in vitro antioxidant activity of leaves and stem of the plant. The antioxidant activities of different concentrations of ethanol extracts of NAT-L and NAT-S were determined by DPPH radical scavenging assay, Reducing power ability, Hydrogen peroxide scavenging assay and Total antioxidant assay. The effective antioxidant activity of NAT-S and NAT-L has found increased with increasing concentration. Comparing NAT-S, there

was an increased activity found in NAT-L extract[62]. The results obtained in the present study indicate that the leaves and stems of Nyctanthes arbor-tristis are a potential source of natural antioxidants.

2.3.7.8. Immuno pharmacological activity

The immuno-pharmacological properties of ethanolic extract of *Nyctanthes arbor-tristis* Linn. (NA) has been investigated. After administration of *Nyctanthes arbor-tristis* in doses of 0.25 and 0.5 g/kg body weight (BW) a significant increase in phagocytic index, leukocyte count and splenic antibody secreting cells were noticed. Stimulation of humoral immune response was further observed with heamagglutination antibody titre. This extract was further submitted to Thin Layer Chromatography (TLC) and High performance liquid chromatography (HPLC) and it confirmed the presence of methoxylated flavonoid quercetin-3, 3'-dimethoxy-7-rhamnoglucopyranose. The results suggested that bio active compound flavonol glycoside of Nyctanthes arbor-tristis influences both humoral as well as cell mediated immune system[63].

CHAPTER - 3

AIM AND OBJECTIVES

Myocardial infarction (MI) is an irreversible necrosis of tissue of a region of myocardium caused by ischemia, which is a perfusion imbalance between demand and supply of blood to the heart via the coronary circulation. There is substantial evidence that ischemic tissue generates oxygen - derived free radical (oxygen radicals). Recently, attention has been focused on non- nutrient phytochemical and Triterpenoids and polyphenols such as the flavonoids, alkaloids, and xanthones derived from different plant species as potential therapeutic agents in the prevention and management of cardiovascular diseases due to their antioxidant nature.

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. *Nyctanthes arbo-tristis* plant contains potential antioxidant phytochemical such as Triterpenoids, polyphenols, flavonoids and related compounds have received increasing attention for their potential role in prevention of human diseases. Phytotherapeutics need a scientific approach to deliver the components in a sustained manner to increase patient compliance and avoid repeated administration. One such novel approach is nanotechnology. Nano-sized drug delivery systems of herbal drugs have a potential future for enhancing the activity and overcoming problems associated with plant medicines.

Hence, integration of the nanocarriers as a novel drug delivery system in the traditional medicine system is essential to conflict more chronic diseases like asthma, diabetes, cancer, CVS disorders and others. The present study is aim to synthesis ZnNPs of *Nyctanthes arbo-tristis* and evaluates the cardioprotective activity against ISO induced myocardial infarction in rats.

OBJECTIVES

- Synthesis and characterization of ZnO-NAT using hydroalcoholic leaf extract of *Nyctanthus arbor-tristis*.
- Evaluation of cardio protective potential of ZnNPs of *Nyctanthes arbo-tristis* by studying its effect on lipid peroxidation, myocyte injury marker (cardiac marker enzymes) and histopathological changes in model of isoproterenol induced myocardial necrosis in rats.

CHAPTER - 5

PLAN OF STUDY

1. Collection and identification of plant material
2. Preparation of Plant extract
3. Synthesis of zinc oxide Nanoparticles.
4. Characterization of synthesized zinc oxide Nanoparticles.
    a) UV-Vis spectroscopy (UV-Vis),
    b) Fourier Transform Infrared Spectroscopy (FTIR),
    c) Transmission Electron Microscopy (TEM)
    d) Scanning Electron Microscopy (SEM)
    e) Power x ray Diffraction Analysis (XRD)
5. Pharmacological study design
    a) Selection of animals
    b) Animal grouping
6. Acute toxicity studies
7. Induction of myocardial infarction using ISO
8. Physical evaluation
    a) Body weight
    b) Feed intake
9. Biochemical estimation
    a) Total cholesterol (TC)
    b) Triglycerides (TG)
    c) High density lipoprotein (HDL)
    d) Low density lipoprotein (LDL)

10. Cardiac markers enzymes

    a) Creatinine phosphokinase (CPK)

    b) Aminotransferase (AST)

    c) Alanine amino transferase (ACT)

    d) Lactate dehydrogenase (LDH)

    e) Alkaline phosphate (ALP)

11. Histopathalogical examination

12. Statistical analysis

CHAPTER-5

MATERIALS AND METHODS

5.1. Drugs and Chemicals

Isoprenaline hydrochloride (isoproterenol) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the chemicals and reagents used in this study were of analytical grade. Propranolol was purchased from the manufacturer Cipla Pharmaceuticals, Mumbai.

5.2. Collection and authentication of plant material

Leaves of *Nyctanthes arbor-tristis* were collected from in and around the region of Namakkal, Tamilnadu, in the month of November. The plant was authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen is preserved in our laboratory for future reference.

5.3. Preparation of plant extract

The plants material was shade dried at room temperature. The dried plant materials were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve no. 40 was used for extraction. Powdered plant material (500 gm) was extracted with 80% methanol at room temperature for 72 hrs. The extract was filtered and concentrated to dryness under reduced pressure and controlled temperature (40^0 C to 50^0 C) in a rotary evaporator until all solvent was removed to give a dark colored molten extract. The percentage yield of the methanolic leaf extract of *Nyctanthes arbor-tristis* was *72%*. The extract was stored in airtight containers in refrigerator maintained below 10^0 C until further use.

5.4. Synthesis of ZnO NPs [64]

1 mM Zinc acetate was dissolved in 50 ml Milli-Q water and

⇩

Stirrer for 1 h respectively.

⇩

20 mL of NaOH solution was slowly added into the Zinc acetate solution

⇩

25 mL of plant extract was added

⇩

The colour of the reaction mixture was changed after 1 h of incubation time.

⇩

Solution was left in stirrer for 3 h Yellow colour appeared after the incubation time confirmed the synthesis of ZnO NPs

⇩

Precipitate was separated and centrifugation at 8000 rpm at 60 °C for 15 min

⇩

pellet was collected and dried hot air oven at 80 °C for 2 h and preserved.

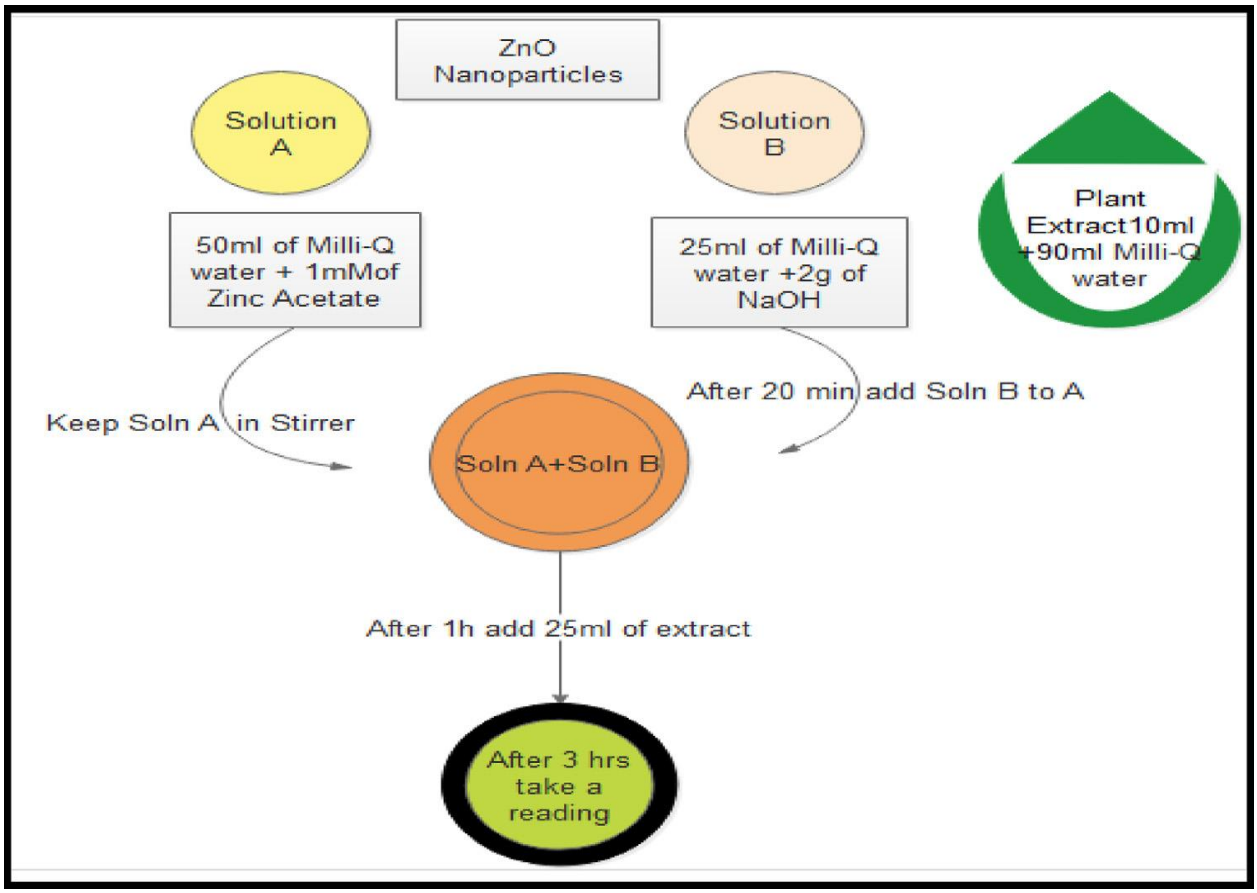


Fig.No:13 Synthesis of ZnO NPs

5.5. CHARACTERIZATION OF ZnO NANOPARTICLES

5.5.1. UV –Visible Spectroscopy

For UV- Visible spectra of synthesized ZnO nanoparticles were re-suspended in equal amount of sterilized de-ionised water and spectrum scans were performed using Shimadzu UV-1800 Spectrophotometer. 2 ml solution of the nanoparticles was taken in quartz cuvette. The scan range was set between 200-800nm and the background was minimised using de-ionised water.

5.5.2. Fourier Transform Infrared (FT-IR) Spectroscopy

The characterisation of phytoconstituents of *Nyctanthes arbor-tristis* hydro alcoholic extract involved in the reduction and stabilization of zinc nanoparticles was investigated by FT-IR analysis (Shimadzu- IR Affinity-1) and the spectra was scanned in the range of 4000-500 cm^{-1} range at a resolution of 4 cm^{-1}.The sample was prepared by grounding the ZnO nanoparticles uniformly in a matrix of dry KBr, compressed to form an almost transparent disc. KBr was used as a standard to analyse the sample.

5.5.3. Transmission Electron Microscopy (TEM)

TEM technique was used to visualize the morphology of the nanoparticles and determination of the size, shape and arrangement of particles. The ZnO nanoparticles was suspended in sterile deionised water, sonicated for 15 min and diluted to yield slightly turbid suspension. The suspension was then coated onto a copper grid and allowed to dry. TEM images were taken on the Philips CM200 7500 model with resolution 2.4 A^0 operating at voltage 20-200kv.

5.5.4. Scanning Electron Microscopy (SEM)

The morphological features of synthesized ZnO nanoparticles from NAT plant extract were studied by Scanning Electron Microscope (JSM-6480 LV). After 24Hrs. of the addition of ZnO the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV.

5.5.5. X Ray Diffraction Analysis (XRD)

The XRD pattern of the synthesized nanoparticles was then recorded using Analytical PW3040/60 XpertPRO model X-ray diffractometer. A thin flim of the sample was made by dipping a glass plate for XRD studies. The instrument was operated at a current of 30mA and voltage of 40KV.The size was calculated using Scherer formula.

$$= \frac{0.94\,\lambda}{\beta \cos\theta}$$

Where,

D = crystal size,

λ = wavelength of X-ray,

Θ = Bragg's angle

β= Full width at half maxima (FWHM) of spectral peak (in radians)

5.6. Evaluation of cardio protective effect

5.6.1. Experimental Animals

The colony inbred female albino wistar Rats , Weighing 150-300gm were obtained from Central Animal house of Swamy Vivekananda college of pharmacy, Elayampalayam, Namakkal -637 205. The animals were kept under standard environmental conditions of 12/12light/dark rhythm, maintained under controlled room temperature (23±2ºC) and a relative humidity of 60%± 10%, in polypropylene cages. They were fed with standard pellet diet and water *ad libitum.* The immature

animals were acclimatized under laboratory conditions three days prior to initiation of the experiment. The cages were cleaned daily by changing the husk bedding.

The experimental protocol was approved by the institutional Animal Ethical Committee (IAEC) of Swamy Vivekananda college of pharmacy, Elayampalayam, Namakkal -637 205.Care and use of laboratory animals were confirmed to CPCSEA guidelines.

IAEC Approval No.: SVCP/IAEC/PG/2/04/2018

5.6.2. Induction of Myocardial Infarction Using Isoproterenol [65]

Myocardial Infarction was induced by dissolving Isoproterenol hydrochloride in normal saline and was injected subcutaneously (s.c.) 85mg/kg body weight for two consecuetive days into rats at an interval of 24 hr to induce experimental MI (14^{th} & 15^{th} day).

5.6.3. Animal grouping

The experimental rats were divided into four groups of six animals each and treated as follows:

Group 1: Normal control animals receiving normal saline (2ml/kg/day) once daily for 15 days.

Group 2: Negative-control animals receiving normal saline (2ml/kg/day) once daily for 15 days and treated with isoproterenol (ISO) (85mg/kg, s.c.) on 14^{th}&15^{th} day.

Group 3: Animals receiving standard drug propranolol (10mg/kg/day) orally 15 days and challenged with isoproterenol (ISO) (85mg/kg, s.c.) on 14^{th}&15^{th} day.

Group 4: Animals receiving hydroalcholic extract NAT–ZnO NP (500mg/kg/day) orally for 15 days and challenged with isoproterenol (ISO) (85mg/kg, s.c.) on 14^{th}&15^{th} day.

Group 5: Animals receiving higher dose NAT –ZnO NP (30 mg/kg/day) orally for 15 days and challenged with isoproterenol (ISO) (85mg/kg, s.c.) on 14^{th}&15^{th} day.

5.7. Acute toxicity studies

Acute toxicity study was conducted in accordance with Organisation for Economic Cooperation and Development (OECD) guidelines for testing of acute oral toxicity (AOT) by up and down procedure (UDP) OECD- No: 425

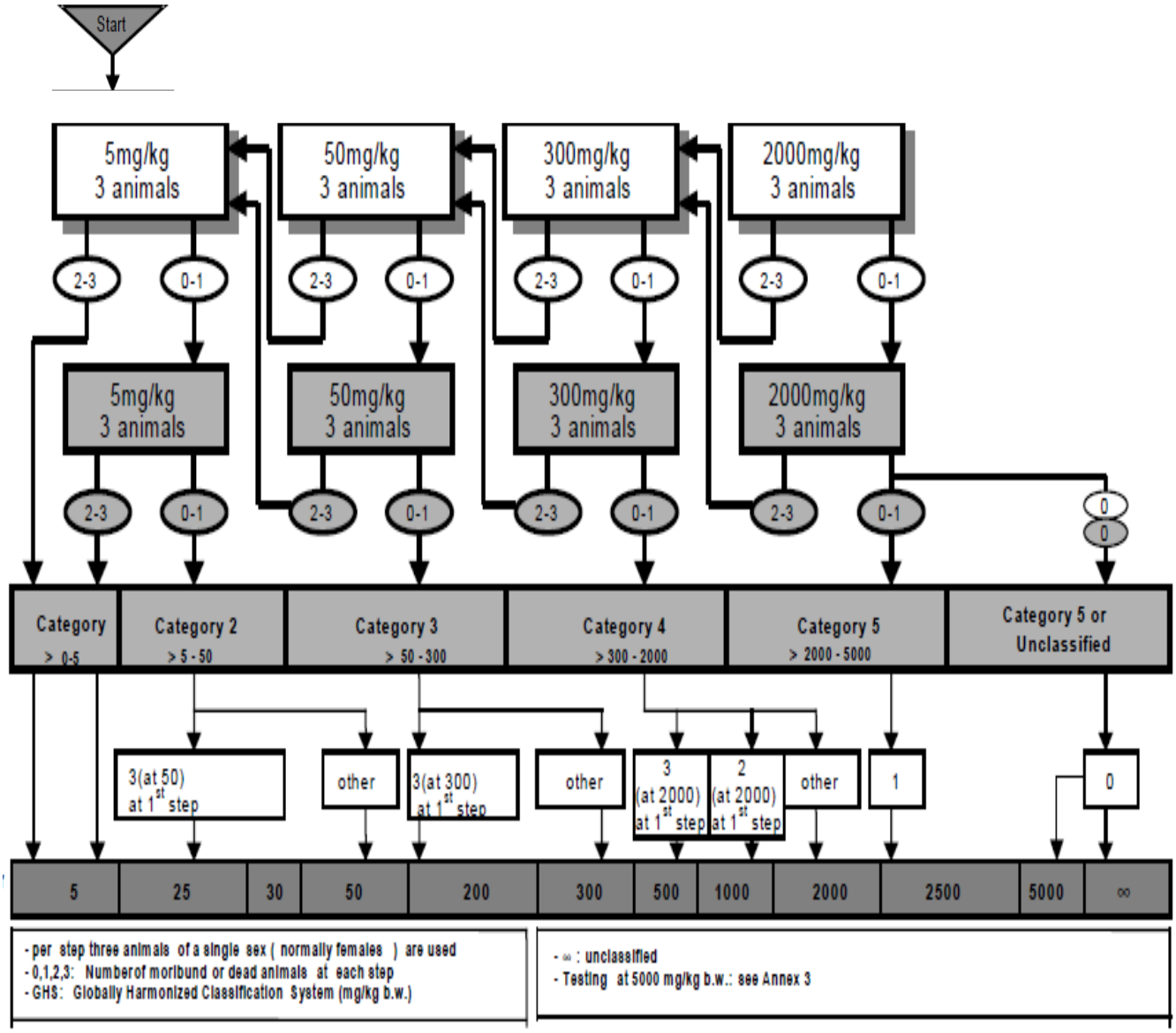


Figure.no:14: OECD Guidelines 425

5.8. Physical evaluation

5.8.1 Measurement of body weight

Body weight of each rats in all groups were measured weekly till end of the treatment using a weighing balance and the changes were recorded.

5.8.2 Measurement of Feed intake

Daily feed consumption was measured in individual treatment groups by using standard weighing balance.

5.9. Biochemical estimation

At the end of experimental period on 14th day rats were fasted overnight (12 h) and blood samples were collected via retro-orbital sinus puncture under mild anaesthesia. Serum was obtained by centrifugation of samples at 3000 rpm for 10 min and used for further plasma lipid profile and cardiac specific injury markers estimations.

5.9.1. Serum lipid profile

Serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and Low density lipoproteins (LDL) were analysed by using commercially available laboratory kits (ARKRAY Healthcare Pvt. Ltd., surat, India).

5.9.2. Serum cardiac specific injury markers

Activity levels of creatine phospokinase-MB (CK-MB), lactate dehydrogenase (LDH), Alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in serum were estimated using commercially available kits (AGAPPE Diagnostics LTD, Kerala, India).

5.10. Histopathological Evaluation

Histological evaluation was performed on lower portion of the heart tissue. Fresh heart tissues were excised and then fixed in 10% formalin for 24 hr. The

fixative was removed by washing through running tap water for overnight. After dehydration through a graded serious of alcohols, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Section were cut into 5 µm thickness and stained with hematoxylin and eosin. After repeated dehydration and cleaning, the sections were mounted and observed under light microscope with 100x magnification for histological changes.

5.11. Statistical analysis

The results of cardio protective activities are expressed as mean ± SEM from four animals from each group. Results were analysed statistically by one way ANOVA followed by post hoc Dunnet's test by using SPSS V.17(student trail version).The difference was considered significant when $p<0.05$.

CHAPTER-6

RESULTS

6.1. CHARACTERIZATION OF ZnO NANOPARTICLES

6.1.1. UV –Visible Spectroscopy

The zinc oxide nanoparticles were efficiently synthesized from *Nyctanthus arbor-tristis* leaf extract with zinc acetate at the ratio of 1:10.With in 30 mins of incubation the dark brown colour changes, an indicator of zinc oxide nanoparticles formation. The green synthesized zinc oxide nanoparticles showed absorbance from 250 to 265 nm and the absorbance centred at 260nm in UV-visible spectroscopy. The UV-visible absorbance spectra result enlightens that the nanoparticles were found to be symmetrical with spherical polydispersed in nature.

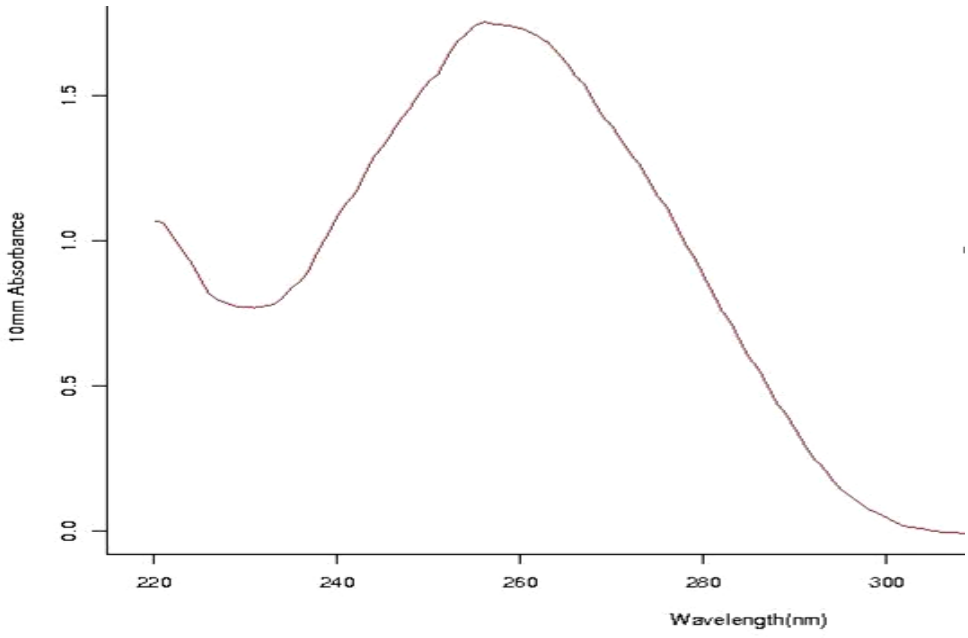


Fig. No.: 15 UV –Visible spectroscopy

6.1.2 Transmission Electron Microscopy (TEM)

TEM analysis was performed in order to investigate the morphological and distribution of our green synthesized zinc oxide nanoparticles.TEM analysis revealed that all the nanoparticles were found in general as spherical shape. The average particle size measured from the TEM images is found to be 50nm

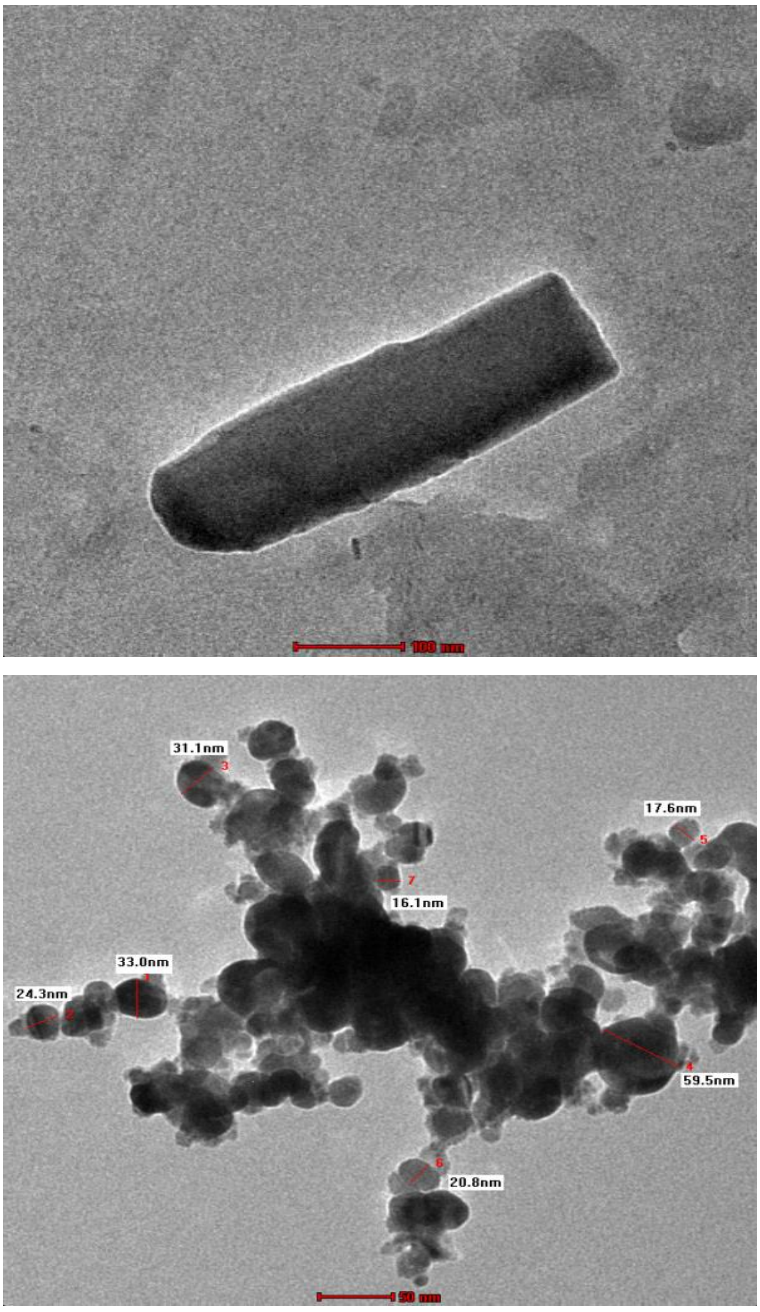
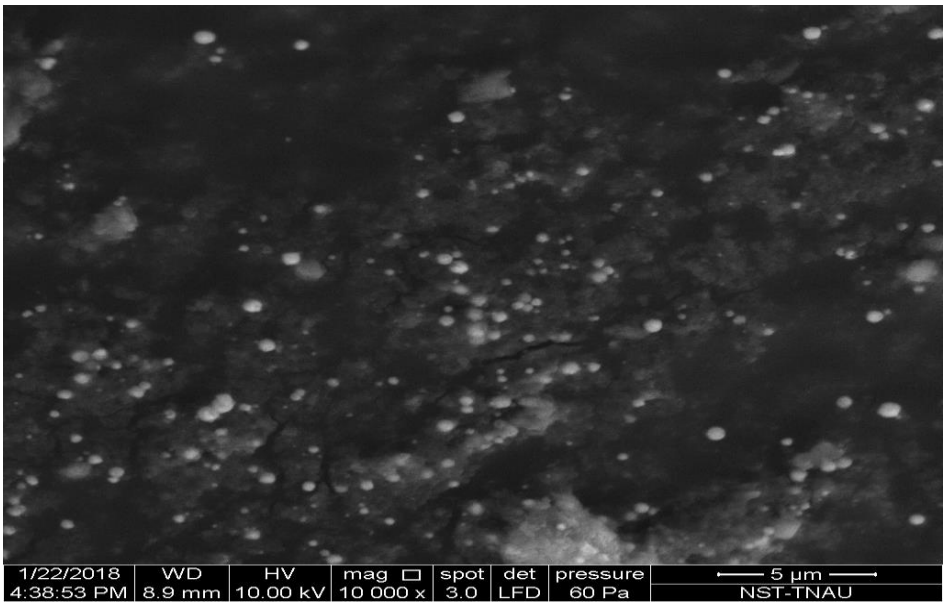


Fig.No: 16.Transmission Electron Microscopy

6.1.3 Scanning Electron Microscopy (SEM)

The analysis of the scanning electron microscopy (SEM) images predicts the formation and the morphology of stable zinc oxide nanoparticles obtained from the current green approach. The images showed the presence of both individual as well as the aggregated ZnONPs. The ZnONPs are mainly uniform spherical shaped with the average range of particle size distribution from 40 nm to 80 nm.



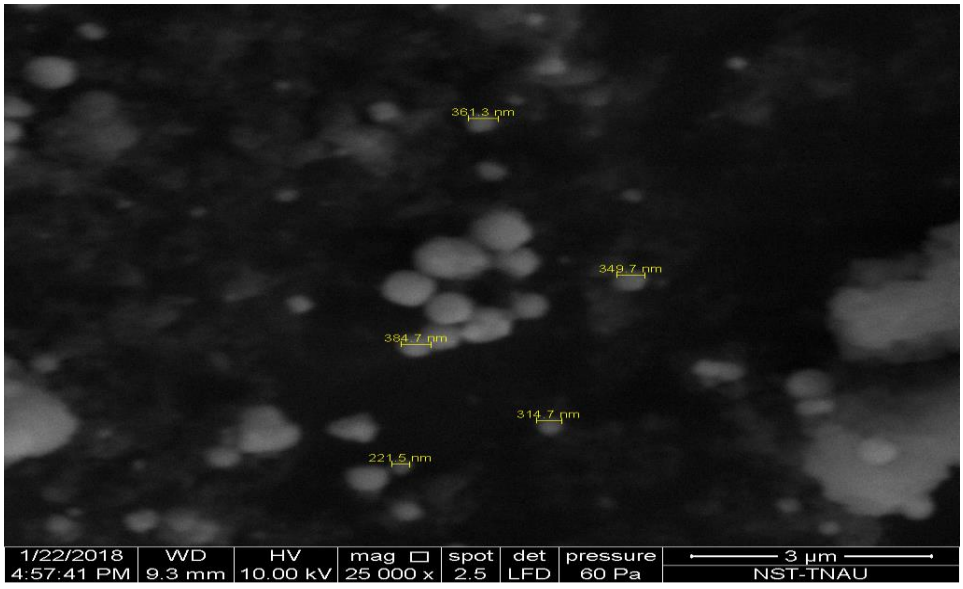


Fig.No:17.Scanning Electron Microscopy (SEM)

6.1.4 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FT-IR) spectroscopy helps establish the identity of various phyto-chemical constituents involved in the reduction and stabilization of the nanoparticles. FT-IR spectrum for dried and powdered ZnO NPs was obtained using Perkin Elmer FT-IR Spectrophotometer Frontier using the technique of Attenuated Total Reflectance (ATR) in the range of 4000–400 cm. The sample for the infrared analysis was carefully prepared to exclude any possibility of the presence of any unbound plant extract residue. The similarities between the spectra with some marginal shifts in peak position, clearly indicate the presence of the residual plant extract in the sample as a capping agent to the ZnO NPs. FT-IR spectroscopy was performed. The FTIR spectra resulted in various peaks at 3262, 2923.56, 1567.84, 1406.82 and 1035.59 cm^{-1}. The peaks at 3262 correspond to H bonded OH stretch and NAH stretch. Peak at 2923.56 corresponds to stretching vibrations of C triple bond C stretch of alkynes. The 1567.84 peak results from the stretching bands of C=O functional groups. The peak at 1406.82 refers to the amine -NH vibration stretch in protein amide linkages The 1035.59 peak results from aromatic amines.

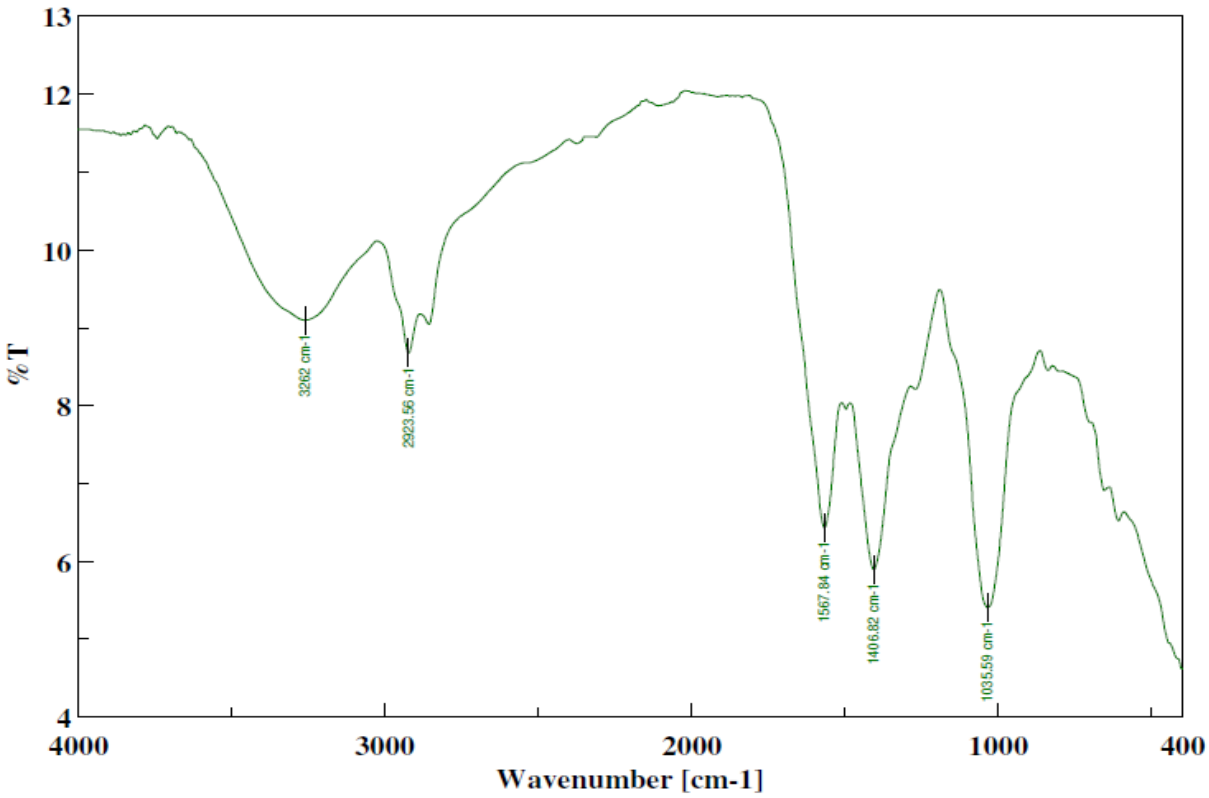


Fig No.: 18. FT-IR Pattern of the ZnONP of *Nyctanthes arbor-tristis* leaf extract

6.1.5 .X ray Diffraction Analysis

Washed and dried sample of ZnO NPs was used for XRD analysis using Ultima IV at the wavelength of 1.5406 A °. XRD was performed in the 2h range of 20–80 degrees at 40 kV and 40 mA with a divergence slit of 10 mm in 2h/h continuous scanning mode. Crystal lattice indices and particle size calculations were performed using the X-ray diffraction pattern of ZnO NPs Diffraction peaks were observed at 2h values of 31.80º, 34.44º, 36.24 º, 47.48 º, 56.62 º, 62.88 º, 66.42 º, 68.0 º and 69.14 º corresponding to lattice planes (100), (002), (101), (102), (110), (103), (200), (112) and (201) respectively. The peaks have been attributed to hexagonal phase of ZnO

Interplanar d-spacing was calculated using Bragg's Law equation

$$2d \sin\theta = n\lambda$$

where,

θ is Bragg's angle of diffraction,

λ is X-ray wavelength, i.e. 1.5406 A ° and n= 1.

Further, particle size was calculated from the intense peak corresponding to (101) plane using Debye–Scherrer formula

$$D \text{ ¼ } 0{:}89k{=}b \cos\theta$$

where

0.89= Scherrer's constant,

k= X-ray wavelength

The value of particle siz was found to be 18.34nm which falls within the size range of 15-48 nm reported by TEM and XRD.

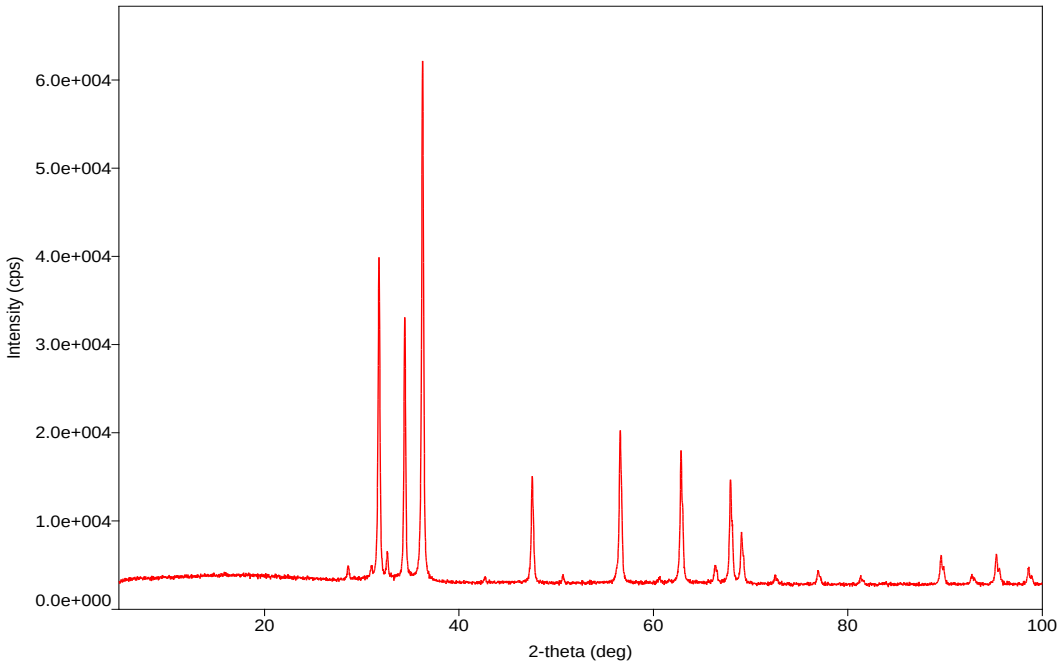


Figure.:19. Power X-Ray Diffraction Analysis

6.2 Changes in body weight

Body weight of the animals of all groups was measured at trial period, initial and a final body weight change in treatment group was compared with control group. Initially there were no significant changes in body weight between treatment groups when compared to the control group. On 15th day weight of ZnO NAT treated group showed significantly increase in body weight compared to other groups.

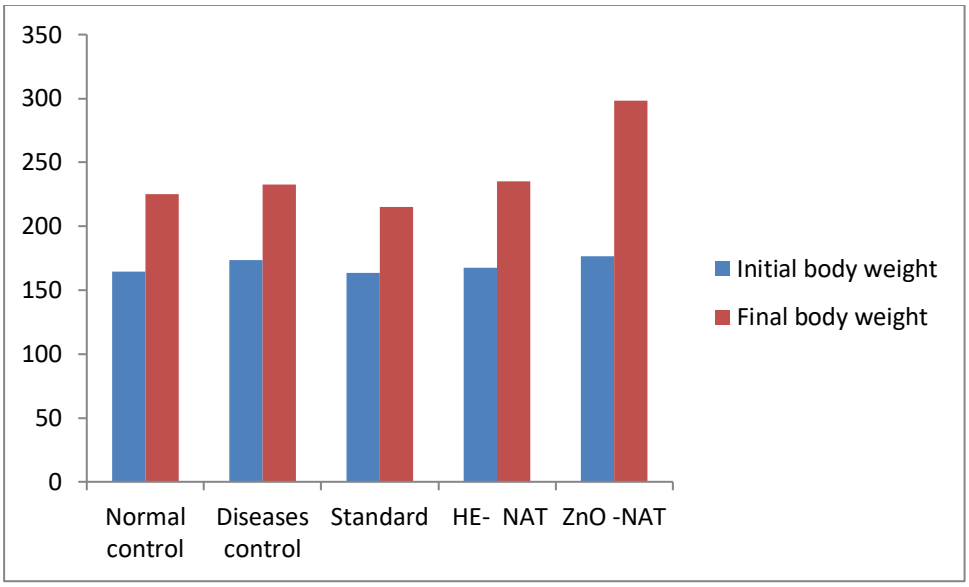
Treatment	Initial body weight(g)	Final body weight(g)
Normal control	164.5± 10.03	225.3±16.26
Diseases control	173.3±12.07	232.4±14.73^{a***}
Standard (Propronolol)	163.6±11.16	215.0±10.57 ^{b***}
HE of NAT(500mg/kg)	167.3±8.73	235.21±6.54 ^{b***}
ZnO NAT (30mg/kg)	176.7±10.23	298.3±15.79 ^{b***}

Table. No:1 Changes in body weight

Values are expressed as mean ± SEM, n=6. Comparisons were made between:

a- Group I vs II, III ,IV and V b- Group II vs I,III ,IV and V

Symbols represent statistical significance: #=P<0.001, $=P<0.01,* = P<0.05



Graph.No:1 Changes in body weight

6.3 Effect of NAT ZnO-NPs on isoproterenol-induced changes in Serum cardiac specific injury markers

The activities of cardiac functional marker enzymes (CPK, LDH, AST, ALT and ALP) in the serum of isoproterenol alone treated group shown significant increases as compare to the normal control group rats. Though the pre treatment of ZnO NPs NAT (50mg/kg) related groups significantly prevented the depletion of myocardial enzymes as compared to the negative control. ZnO NPs NAT (50mg/kg) treatment, however, did not show any significant effect on the activity of ALP enzyme.

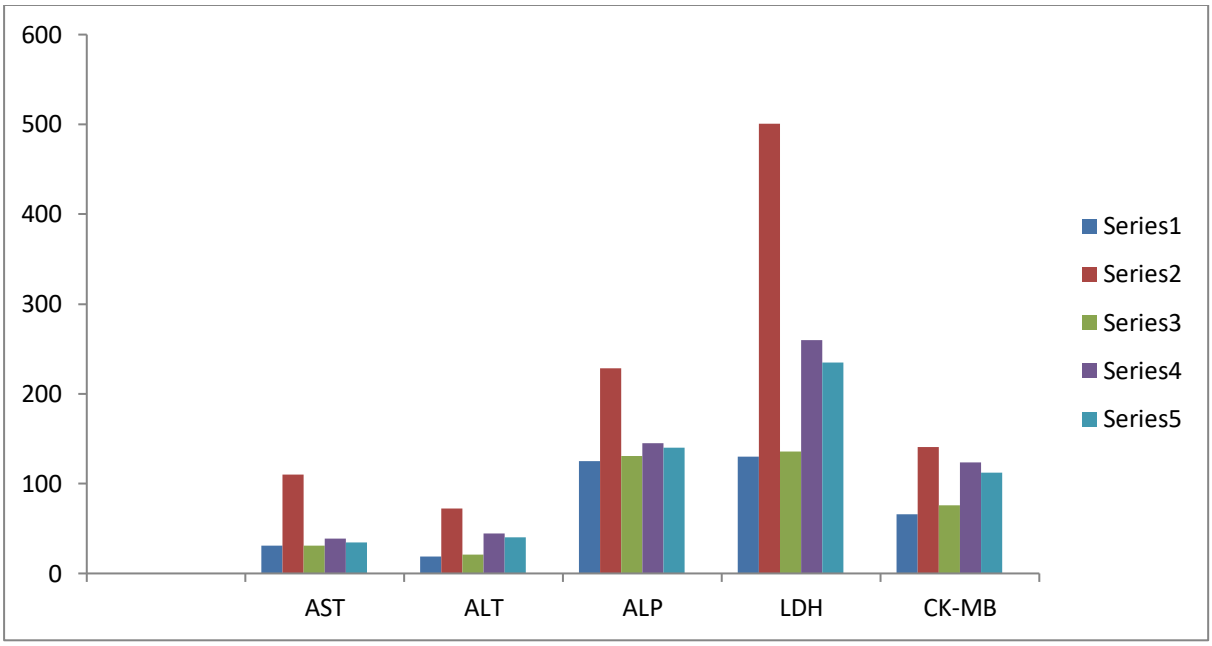
Table.No :2 Effect of HENA on Marker enzymes of Rats Myocardial serum

Group	AST	ALT	ALP	LDH	CK-MB
Normal control	17.01±1.48***	15.56±2.25***	95.20±3.14**	213.2±12.59***	65.95±15.04
Diseases control	50.02±4.25***	52.07±3.17***	228.7±9.05***	328.9±16.63***	140.6±4.45
Standard	50.80±1.05 a	33.01±1.05 a#	130.9±7.09 a# a#	269.46±11.09 a#	76.08±1.02
HE of NAT(500mg/kg)	39.06±2.34***	49.42±3.15b***	145.04±3.15a**	298.09±1.64 ***	123.35±9.08
ZnO NAT (30mg/kg)	16.9±1.08 b***	22.36±1.36 b***	118.78±2,08 b*	210.8±2.08 b***	112.06±1.67

Values are expressed as mean ± SEM, n=6. Comparisons were made between:

a- Group I vs II, III ,IV and V b- Group II vs I,III ,IV and V

Symbols represent statistical significance: #=P<0.001, $=P<0.01,* = P<0.05



Graph.No :2 Effect of HENA on Marker enzymes of Rats Myocardial serum

6.4 Effect of ZnO NP-NAT on isoproterenol- induced changes in serum Lipid profile

The results of ZnO NPs NAT on isoproterenol induced changes in serum lipid profile are cited in Table 2. The level of serum total cholesterol, triglycerides, LDL significantly increases and decrease in the levels of HDL in isoproterenol treatment as compartment to control group. The treatment ZnO NPs NAT of for 15 days however, significantly restored the lipid profile to near normalcy as compared to the negative control.

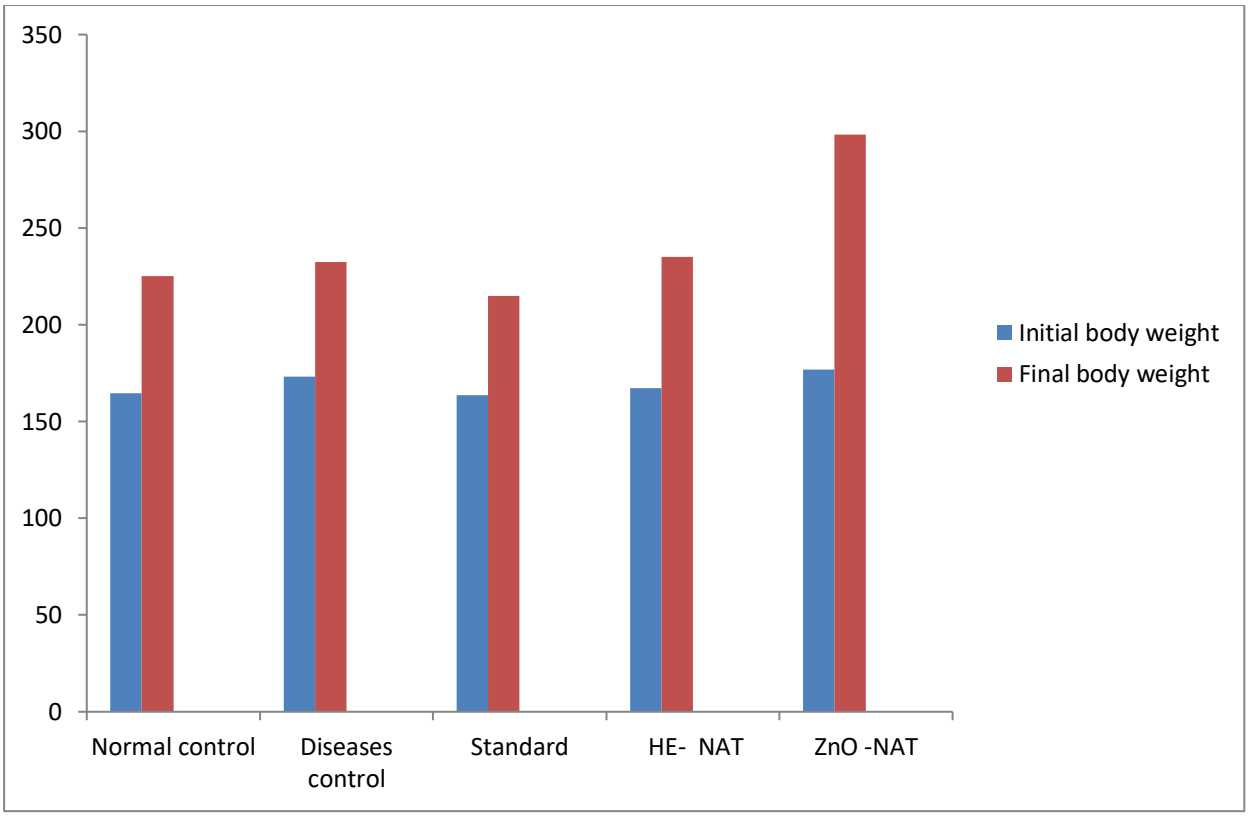
Table.No :3 Effect of ZnO NP-NAT on isoproterenol- induced changes in serum Lipid profile

Treatments	TC (mg/dl)	TG(mg/dl)	HDL(mg/dl)	LDL(mg/dl)
Normal control	108.17 ± 4.98	95.67 ± 4.09	49.33 ± 5.38	99.83 ± 4.71
Diseases control	177.67 ± 8.11a$^{\#}$	144.50 ± 5.46 a$^{\#}$	33.17 ± 3.99 a*	128.83 ± 7.85 a*
Standard	169.5 ±5.32	134.42 ±7.01	33.17 ±5.87	119.6 ±4.23
HE of NAT(500mg/kg)	127.31 ± 7.18a* b$^{\#}$	115.83 ± 6.98 a* b$^{\$}$	38.00 ± 4.03	112.66 ± 7.01
ZnO NAT (30mg/kg)	108.24 ± 6.18 b$^{\#}$	96.00 ± 6.92 b$^{\#}$	48.67 ± 5.00 b*	97.17 ± 5.71 b*

Values are expressed as mean ± SEM, n=6. Comparisons were made between:

a- Group I vs II, III and IV. And V b- Group II vs III and IV,V

Symbols represent statistical significance: #=P<0.001, $=P<0.01,* = P<0.05

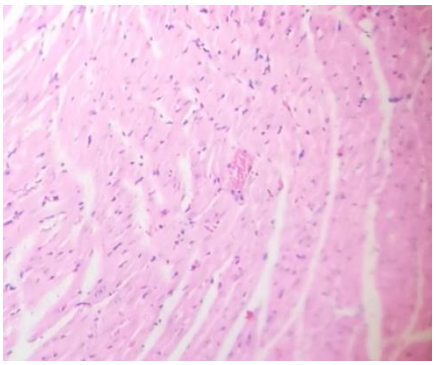


Graph.No:3 Effect of ZnO NP-NAT on isoproterenol- induced changes in serum Lipid profile

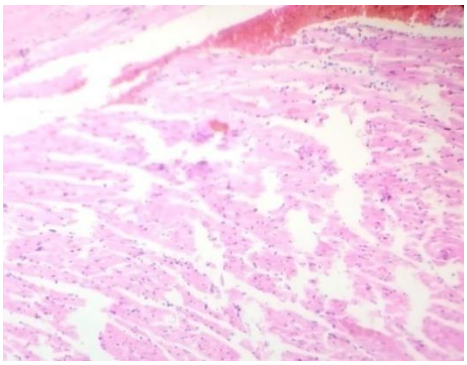
6.5 Histopathological observations

Histopathological examination of myocardial tissue from normal control animals exhibited clear integrity of myocardial membrane. Histopathological findings confirmed the induction of myocardial infarction by isoproterenol. Heart tissues from isoproterenol treated rats showed widespread myocardial structure disorder and sub endocardial necrosis with capillary dilatation and leukocyte infiltration as compared to normal control rats. Treatment of hydroalcoholic extract of NAT 500mg/kg showed mild muscle separation and few inflammatory cells, lower dose of 50mg/kg treatment showed no change in histo-architecture of heart tissue as compared to normal control rats.

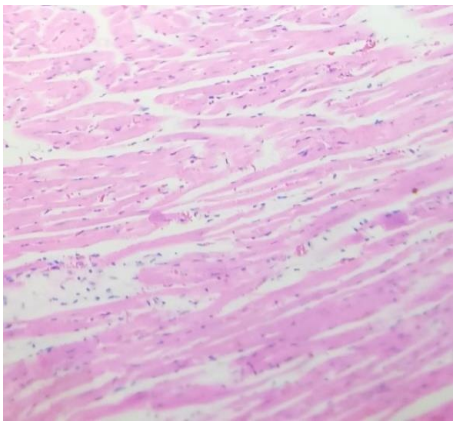
A) Control



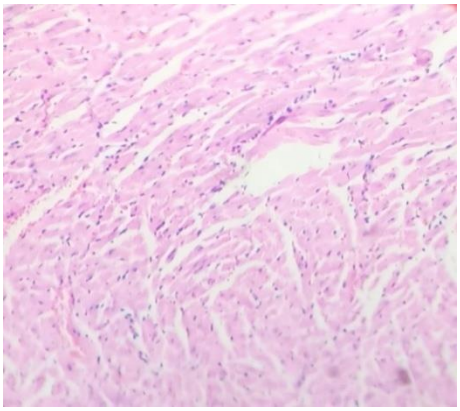
B) Diseases control



C) Standard



D) HE-NAT



E) ZnO NAT

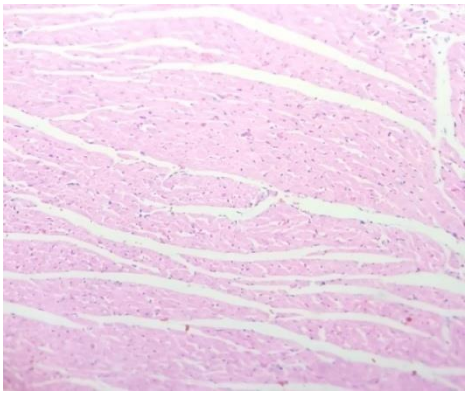


Fig.,20. Histopathological Examination

CHAPTER-7

DISCUSSION

zinc oxide nanoparticles (ZnO- NP) as a material of low toxicity, because zinc is an essential trace element in the human body and is commonly present in foods or added as a nutritional supplement, so zinc attracts little attention towards assessment of toxicity using nanoparticles. The fact that zinc appears to have protective effects in coronary artery diseases and cardiomyopathy is attributed to its critical role in redox signalling pathway, whereby certain triggers such as ischemia and infarction leads to release of zinc from proteins and cause myocardial damage[2] In that the plant extract of nyctanthus arbor-tristis is synthesised in the form of nanoparticles.

IN UV-Visible ,the zinc oxide nanoparticles were efficiently synthesized from *Nyctanthus arbor-tristis* leaf extract with zinc acetate at the ratio of 1:10.With in 30 mins of incubation the dark brown colour changes, an indicator of zinc oxide nanoparticles formation. The green synthesized zinc oxide nanoparticles showed absorbance spectra at 260nm in UV-visible spectroscopy. The UV-visible absorbance spectra result enlightens that the nanoparticles were found to be symmetrical with spherical polydispersed in nature.

TEM analysis was performed in order to investigate the morphological and distribution of our green synthesized zinc oxide nanoparticles.TEM analysis revealed that all the nanoparticles were found in general as spherical shape. The average particle size measured from the TEM images is found to be 50nm. The analysis of the scanning electron microscopy (SEM) images predicts the formation and the morphology of stable zinc oxide nanoparticles obtained from the current green approach. The images showed the presence of both individual as well as the aggregated ZnONPs. The ZnONPs are mainly uniform spherical shaped with the average range of particle size distribution from ...nm.

Fourier transform infrared (FT-IR) spectroscopy helps establish the identity of various phyto-chemical constituents involved in the reduction and stabilization of the nanoparticles.FT-IR spectrum for dried and powdered ZnO NPs was obtained using Perkin Elmer FT-IR Spectrophotometer Frontier using the technique of Attenuated

Total Reflectance (ATR) in the range of 4000–400 cm. The sample for the infrared analysis was carefully prepared to exclude any possibility of the presence of any unbound plant extract residue. The similarities between the spectra with some marginal shifts in peak position, clearly indicate the presence of the residual plant extract in the sample as a capping agent to the ZnO NPs. FT-IR spectroscopy was performed. The FTIR spectra resulted in various peaks at 3340.6, 3258.2, 2127.8, 1641.1, 1456.7, 1362.5, 1040, 1026.8, 746.25, 620.65 cm^{-1} The peaks at 3340.6 and 3258.2 correspond to H bonded OH stretch and NAH stretch. Peak at 2127.8 corresponds to stretching vibrations of C „ C stretch of alkynes. The 1641.1 peak results from the stretching bands of C,O functional groups . The peak at 1456.7 refers to the amine -NH vibration stretch in protein amide linkages The 1362.5 peak results from aromatic amines and the two peaks at 1040 and 1026.8 from C-N stretch of aliphatic amines. The 746.25 and 620.65 peaks correspond to alkanes and supposedly, C-H bend in alkynes.

In Xray diffraction, ZnO NPs was used for XRD analysis using Ultima IV at the wavelength of 1.5406 A °. XRD was performed in the 2h range of 20–80 degrees at 40 kV and 40 mA with a divergence slit of 10 mm in 2h/h continuous scanning mode. Crystal lattice indices and particle size calculations were performed using the X-ray diffraction pattern of ZnO NPs Diffraction peaks were observed at 2h values of 31.80, 34.44, 36.24, 47.48, 56.62, 62.88, 66.42, 68.0and 69.14 corresponding to lattice planes (100), (002),(101), (102), (110), (103), (200), (112) and (201) respectively. The peaks have been attributed to hexagonal phase of ZnO.

Many of today's diseases including cardiac diseases have been linked to oxidative stress which is initiated by the reaction of free radicals with biological macromolecules such as proteins, lipids and DNA. Generally antioxidants, preferably from natural sources, have been considered as effective treatments.

Isoproterenol produces relative ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension and induce myocardial ischemia due to cytosolic Ca2+ overload The oxidative stress may be exerted through quinone metabolites of isoproterenol, which reacts with oxygen to produce ROS and interfere with glutathione reductase), superoxide dismutase and ATP pumps .

Oleanolic acid is a lipophilic *β*-blocker in nature. *β*-Adrenergic blockers have long been useful adjuvants in the management of myocardial ischemic

syndromes. The myocardial necrosis observed in the animals receivingisoproterenol can also be attributed to peroxidative damageas it has been previously reported that isoproterenol generateslipid peroxides.

ISO induced myocardial necrosis has been reported to alter membrane permeability and to cause leakage of marker enzymes of cardiac damage (LDH, CK-MB, AST, ALT and ALP) into the blood stream.[81] Significantly elevated levels of these marker enzymes have been recorded in IP induced myocardial damage.[82] Result of this study assures that significant elevated level of marker enzymes LDH, CK-MB, AST, ALT and ALP in IP alone treated group as compared to the normal control. On the contrary, ZnO NP NAT treatment protected the structure and functional integrity of myocardial membrane as evident from the significant reduction in the elevated levels of these serum marker enzymes in the rats when compared to the isoproterenol treated rats. Increase in the activity of these enzymes is diagnostic indicators of myocardial infarction[83] and are indicative of cellular damage and loss of functional integrity of cell membrane.[84] The reversal of these enzyme activities by pretreatment with ZnO NP NAT indicates its therapeutic potential against myocardial infarction in a dose dependent manner by preventing the ISO induced leakage of marker enzymes.

Histopathological examination of myocardial tissue in control illustrated clear integrity of the myocardial cell membrane and no inflammatory cell infiltration was observed. Isoproterenol injected rats showed coagulative necrosis, separation of cardiac muscle fibres and infiltration of inflammatory cells. The reduced inflammatory cell infiltration and normal cardiac muscle fibre architecture further confirmed the cardioprotective effect of ZnO NP NAT in a dose dependent manner.

From the present study it is clear that the ZnO nanoparticles in Nyctanthus arbor tristis shows the significant cardioprotective activity at both doses against ISO induced myocardial infarction it is difficult to establish the mechanism of action for cardioprotection against induced myocardial infarction but the presence of Phytochemicals such as oleanoic acid,alkaloids,Flavanoids, Saponin, Amino acid may plays a vital role against ISO induced myocardial damage by its cardioprotective activity.

CHAPTER - 8

SUMMARY AND CONCLUSIONS

Myocardial infarction occurs when there is imbalance between coronary blood supply and myocardial demands which results in the acute condition of necrosis of myocardium. MI is the common presentation of the ischemic heart disease. Even though clinical care is improved, public awareness is raised and health innovations are widely used, myocardial infarction still remains the leading cause of death worldwide. In our study isoproterenol was used to induce MI in rats, because of this pathological changes mimics the human MI.. Hence in our study methanolic leaf extract of ZnO NAT was used for evaluating the *in vivo* cardioprotective activity against isoproterenol induced MI.

The treatment with ZnO NAT successfully restored serum lipid profile i.e. the elevated triglycerides, LDL-cholesterol, total cholesterol levels and also increases the HDL cholesterol levels at a dose dependent manner against the isoproterenol induced MI.

And also result of this study indicate that the pretreatment of ZnO NAT prevent the elevation of cardiac activity marker enzymes LDH, CK-MB, AST, ALT and ALP against IP induced MI. It might be indicates the cardioprotective effect of *Nyctanthus arbor tristis.* Further histopathological analysis result conform the significant cardioprotective effect of hydro alcoholic leaf extract of *Nyctanthus arbor tristis* against IP induced MI.

Nyctanthes arbo-tristis plant contains potential antioxidant phytochemical such as Triterpenoids, polyphenols, flavonoids and related compounds have received increasing attention for their potential role in prevention of human diseases. Phytotherapeutics need a scientific approach to deliver the components

in a sustained manner to increase patient compliance and avoid repeated administration. One such novel approach is nanotechnology. Nano-sized drug delivery systems of herbal drugs have a potential future for enhancing the activity and overcoming problems associated with plant medicines. ZnO NAT is a more potent compared to Hydroalcoholic extract.

Based on the present study it could be concluded that subcutaneous injections of isoproterenol induced myocardial infarction in rats as identified by the release of myocyte injury markers and altered lipid profile in serum. Cardioprotective effect of ZnO NAT was proved by reduction in cardiac marker enzymes, altered lipid profile and histopathological studies. In comparison to the hydro alcoholic extract ZnO nano particle of NAT treatment poses more potent cardioprotective effect. Further investigation is needed to explore the active principles and exact mechanism of action of *Nyctanthus arbor tristis* plant in prevention and treatment of cardio vascular disorders.

CHAPTER- 9

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