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Cardioprotective activity of chitosan-*Terminalia arjuna* extract nanoparticles against lead acetate induced cardiac cell damage in rat

Gomathi Periyasamy[1], Madhusudan Sudhakarrao Bele[2],Kavita Khatana[3], Ahmad Lalahmad Shaikh[4], Shankarappa Kambhoja[5],Shivam Lale[6], Smita P Wasnik[7], Adeep Kujur[8],Meesala Sudhakar[9],Chandore Hemant Dnyaneshwar[10], Arpan Kumar Tripathi[11]

1. Professor, School of Pharmacy, Guru Nanak Institutions Technical Campus, Ibrahimpatnam, Ranga Reddy (dt), TelanganaPin: 501506
2. Assistant Professor Department of Zoology, Shri Vasantrao Naik Mahavidyalaya Dharni Dist.Amravati 444702
3. Postdoctoral Fellow, Chemical Engineering Department, Shiv Nadar Institution of Eminence Deemed to be University, Pin- 201314
4. Assistant Professor, Department of Zoology, Shri Vasantrao Naik Mahavidyalaya DharniAt. PO. Kusumkot(B), Dharni, Tq. Dharni, Dist. Amravati (M.S.) 444702.
5. Professor,Shri Devi Institute of Pharmaceutical Sciences,Thumkur, Karnataka – 572 106, India
6. Associate Professor/HoD (Pharma. Chemistry), MET's Institute of D Pharmacy, Bhujbal Knowledge City, Adgaon, Nashik.Pin: 422003
7. Associate professor, Faculty of Pharmacology, Valmik Naik College of Pharmacy AtTelwadi, Tal. KannadDist Aurangabad. Maharashtra 431003
8. Assistant Professor, University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh
9. Guest Lecturer, Department of Microbiology&Bioinformatics Department,Atal Bihari Vajpayee Viswavidyalayan, Bilaspur, Chhattisgarh, India
10. Head and Assistant professor, Department of Horticulture, Shikshan Maharshi DayanandeoMohekar Mahavidyalaya Kalamb, Affiliated underDr. Babasaheb Ambedkar Marathwada University, Chatrapati Sambhajinagar
11. Associate Professor, Shri Shankaracharya Professional University, Junwani, Bhilai, Chhattisgarh, India

Corresponding Author
Dr. Gomathi Periyasamy
Professor
School of Pharmacy, Guru Nanak Institutions Technical Campus, Ibrahimpatnam, Ranga Reddy (dt), Telangana
Pin : 501506
Email: pgoms@yahoo.com

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Abstract: This paper investigates the use of nanoparticles composed of Chitosan and Terminalia arjuna extracts for protecting cardiac cells from lead acetate-induced damage in rats. The Chitosan-Terminalia arjuna extract nanoparticle was analyzed using Dynamic Light Scattering (DLS) and scanning electron microscopy (SEM). The study involved three groups of 100 rats each: one group received only purified water, another group received intraperitoneal injections of lead acetate (15 mg/kg BW), and a third group received either lead acetate injections or oral doses of Chitosan-Terminalia arjuna nanoparticles at 100, 200, or 400 mg/kg BW. Blood samples were collected to measure concentrations of creatinine kinase-MB (CK-MB) and lactate dehydrogenase (LDH). Additionally, levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), malondialdehyde (MDA), and heart damage were evaluated histologically in cardiac tissues. SEM analysis revealed that the Chitosan-Terminalia arjuna extract nanoparticles had a rough surface and irregular shape, with a size of 312.9 ± 25.7 nm as determined by DLS. Lead acetate exposure resulted in increased levels of LDH, CK-MB, and MDA, while it decreased SOD and GPx levels. Histological assessments confirmed the lead acetate-induced damage to cardiac cells. Regardless, at 400 mg/kg BW, a nanoparticle comprising Terminalia arjuna extract and Chitosan reduced LDH, CK-MB, and MDA levels while boosting SOD and GPx. Plus, the nanoparticle with 400 mg/kg BW of chitosan and Terminalia arjuna extract damaged cardiac cells more extensively overall. The findings provide credence to the theory that the Chitosan-Terminalia arjuna nanoparticle shields rats from lead acetate-induced cardiotoxicity by virtue of its potent antioxidant characteristics.

Keywords: Chitosan-Terminalia arjuna extract nanoparticle, Lead acetate, Cardiac cells, LDH, CK-MB, MDA, Antioxidant.

I. Introduction:

Global environmental degradation and heavy metal exposure are growing problems. Lead acetate is one needlessly dangerous heavy metal that can cause extensive damage to many biological tissues [1]. There is a correlation between lead poisoning and cardiovascular disease. Since the latter lowers antioxidant levels and leads the heart to produce more reactive oxygen species (ROS), it renders the system more sensitive to oxidative stress. One possible diagnostic of heart cell injury is malondialdehyde (MDA), an optional consequence of lipid peroxidation that may be used because to an increase in free radicals [2].

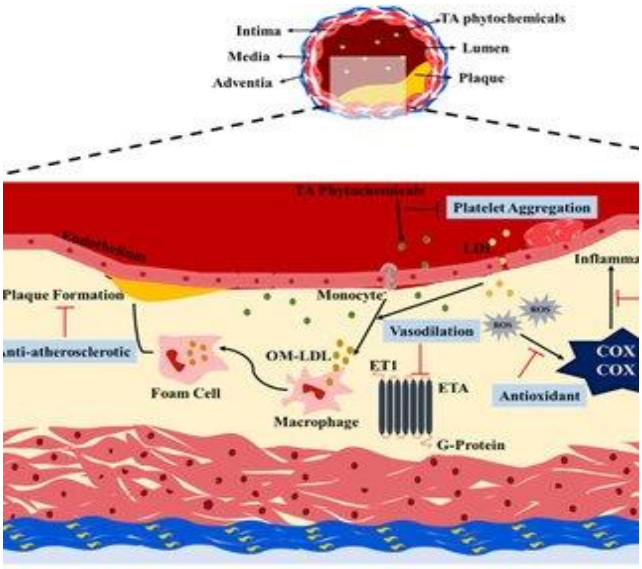

Figure 1: Terminalia arjuna

Recent research has demonstrated the protective effects of commonplace objects and natural medications against lead-induced oxidative damage and premature ageing. Commonplace products having antioxidant qualities, such as chitosan-Terminalia arjuna, can help reduce the damage that free radicals cause to cardiac cells. These compounds are potent antibacterial agents, strong free radical scavengers, vasodilatory, hostile to hypersensitive, mitigating, cardioprotective, safe, energizing, antiviral, and estrogenic exercises.

Regular things can be used to create normal item nanoparticles, which have gained attention recently due to their advantages in terms of bioavailability, dissolvability, and survivability. Chitosan is a commonly used regular substance in pharmaceutical and biological applications; it is well-known for its antimicrobial, antioxidant, anti-HIV, anti-hyperlipidemia, anti-diabetic, relaxing effects, drug delivery, and resistant-upgrading qualities. This review set out to examine the hypothesis that a nanoparticle containing Chitosan-Terminalia arjuna extract would protect heart cells from lead exposure in albino Wistar rats [3].

II. Cardiovascular Diseases:

Globally, CVDs cause a startling number of deaths annually, more than any other cause. These illnesses are common everywhere, impacting people of various ages, socioeconomic backgrounds, and geographic areas. They are not specific to any one area or population.

Figure 2: Cardiovascular diseases

Plaque accumulation narrows the coronary arteries, lowering blood flow to the heart muscle and causing coronary artery disease (CAD), the most prevalent cause of CVD. If the heart's blood supply is totally cut off, this illness, which frequently presents as angina (chest pain), can result in heart attacks. Another common CVD is stroke, which happens when there is an interruption in blood flow to the brain, either because of a clot (ischemic stroke) or bleeding (hemrhagic stroke). This can cause significant neurological deficits and brain damage [4].

High blood pressure, or hypertension, is a major contributor to CVDs because it gradually increases the workload on the cardiovascular system. Peripheral artery disease does more than only make you sick; it also reduces blood flow to your extremities, which can lead to pain and even heart attacks and strokes.

CVDs affect not only an individual's health but also the social and economic spheres. These illnesses lower the general quality of life for afflicted people and their families, place a heavy financial burden on healthcare, and reduce productivity owing to early mortality and impairments. A multimodal strategy is needed to address the burden of CVDs. This strategy should include public health campaigns to encourage healthy lifestyles, routine screenings for early diagnosis, access to reasonably priced and efficient treatments, and continuous research to expand medical knowledge and treatment options [5].

In conclusion, cardiovascular illnesses continue to pose a serious threat to world health, necessitating coordinated action from communities, legislators, and healthcare professionals. We can lessen the effects of CVDs and work towards a healthy future for everybody by putting an emphasis on prevention, expanding access to healthcare, and encouraging innovation in cardiovascular research and treatment [6].

i. Types of Cardiovascular Diseases:

Heart failure, in which the heart fails to pump blood efficiently, resulting in symptoms like fatigue and fluid retention; (CAD), which restricts blood flow to the heart and may cause angina or heart attacks; Stroke, which is characterised by disruptions in brain blood flow resulting in varying degrees of brain damage; Hypertension, a persistent elevation in blood pressure that increases the risk of heart attacks and strokes; and (PAD), which narrows limb arteries and heightens the risk of cardiovascular events.

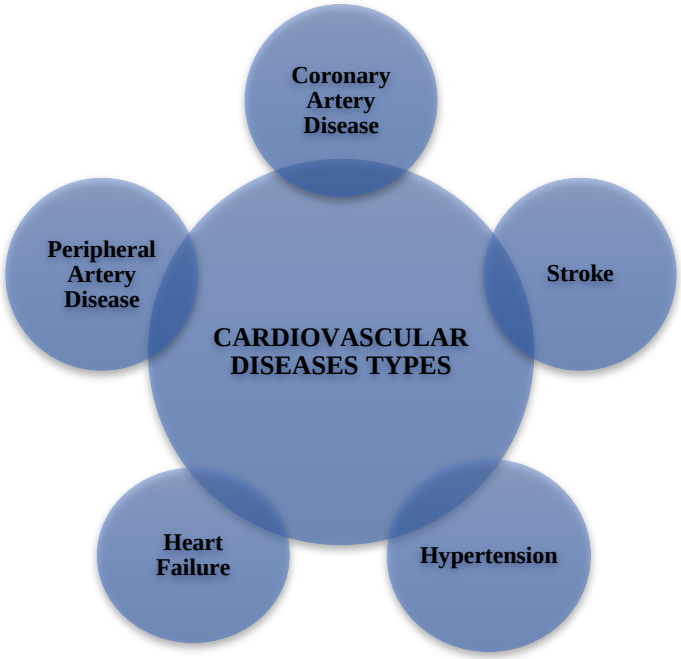

Figure 3: Different Types of Cardiovascular Diseases

All of these circumstances highlight how difficult and urgent it is to treat CVDs in order to improve public health outcomes throughout the world [7]. Heart and blood vessel disorders are the broad category of ailments that make up cardiovascular diseases. Important categories consist of:

- **Coronary Artery Disease (CAD):** affects the arteries that supply blood to the heart muscle, often leading to heart attacks and angina.
- **Stroke:** Brain injury may result from an interruption in the blood supply to the brain. Hemorrhagic strokes are one type that happen when blood clots obstruct blood flow to the brain.
- **Hypertension (High Blood Pressure):** chronically elevated blood pressure, a major risk factor for heart attacks, strokes, and other cardiovascular issues.
- **Heart Failure:** Involves the body accumulating fluid and experiencing weariness and shortness of breath due to the heart's ineffective blood pumping [8].
- **Peripheral Artery Disease (PAD):** Limb-specific artery narrowing, which can frequently result in leg pain and raise the risk of heart attack and stroke [9].

ii. Prevalence of Cardiovascular Diseases:

Globally, cardiovascular disorders are frighteningly common and impact people of all ages and socioeconomic backgrounds:

- **Global Impact:** Deaths from cardiovascular diseases (CVDs) exceed 17.9 million annually, making them the leading cause of mortality on a global scale.
- **Regional Variances:** Although CVDs impact people everywhere, there are notable regional variations in risk factors and prevalence [10]. Countries with lower and moderate-income levels are disproportionately affected since they have less access to healthcare and a greater rate of risk factors like obesity, smoking, and poor diets.

iii. Impact on Global Health:

Cardiovascular diseases (CVDs) have a significant influence on global health, affecting economies and cultures all over the world in addition to personal health [11]. The financial cost

of CVDs is enormous; it includes high medical expenses, lost productivity, and a large number of disability-adjusted life years (DALYs). Furthermore, those who struggle with CVDs frequently experience a reduced quality of life as a result of enduring symptoms, possible consequences, and the requirement for continuous medical care[12].

Figure 4: Impact On Global Health

Comprehensive public health programmes that put an emphasis on early detection, prevention, and efficient treatment methods are required to address these issues. Global efforts seek to enhance global health outcomes and lessen the ubiquitous influence of CVDs by addressing risk factors and improving patient education [13].

Cardiovascular disorders have an impact on economies and communities worldwide, far beyond its effects on an individual's health:

- **Economic Burden:** Due to medical expenses, lost productivity, and disability-adjusted life years (DALYs) lost, CVDs have a significant financial impact.
- **Quality of Life:** People who have cardiovascular diseases (CVDs) frequently have lower quality of life because of symptoms, complications, and continuous medical care.
- **Public Health Challenge:** To reduce risk factors and enhance outcomes, treating CVDs calls for all-encompassing approaches that include patient education, early detection, treatment, and prevention [14].

The frequency and impact of cardiovascular illnesses provide a serious threat to world health, requiring coordinated efforts from several sectors and specialties. We can work towards a future in which fewer lives are lost to these treatable and avoidable disorders by increasing knowledge, encouraging healthy lifestyles, and improving healthcare systems. Let's work together to promote heart health and make sure that hearts remain strong and stable everywhere [15].

III. Materials and method:

i. Chemicals:

From Perfect Analytical Services in Raipur, Chhattisgarh, lead acetate was acquired. The MDA in tissues assay kit was chosen for the study. Assay kits were used to measure the tissue levels of SOD and GPx activities.

ii. Preparation of Chitosan-Terminalia arjuna Nanoparticles:

These nanoparticles of chitosan and Terminalia arjuna extract were made via ionotropic gelation. The chitosan solution was filtered after being made with 0.1% (v/v) glacial acetic acid and 0.2% (w/v).

Tripolyphosphate (TPP) was dissolved to a concentration of 0.1% (w/v) in deionized water to create the solution. After adding 0.4% (w/v) Terminalia arjuna extract to a 0.2% (w/v) chitosan solution in 70% ethanol and stirring continuously, the mixture was sonicated for five minutes.

Moreover, TPP solution was added dropwise while being constantly stirred. The TPP solution to chitosan ratio was kept at 2:1. Following a 20-minute centrifugation at 25,000 rpm for the supernatant, the chitosan-Terminalia arjuna sediment was examined.

iii. Characterization of Nanoparticles by Scanning Electron Microscopy and Dynamic Light Scattering:

SEM was used to examine the surface morphological characteristics of the Chitosan-Terminalia arjuna extract nanoparticle, including particle size, shape, and topography. 2.2 Malvern Instruments was used for dynamic light scattering. The Chitosan-Terminalia arjuna extract nanoparticle's average particle size was ascertained.

iv. Experimental Animal:

The majority of the research took place in KIPS, Bhilai, Chhattisgarh, India, utilizing male Wistar rats weighing between 200 and 250 grams (2.5–3 months old).

v. Experimental Design:

100 male rodents were partitioned into three gatherings, and each gathering was given the accompanying infusions: refined water (control bunch), lead acetic acid derivation (15 mg/kg BW), and chitosan-Terminalia arjuna nanoparticles. An hour after the rodents were given the chitosan-Terminalia arjuna remove nanoparticles on the fourth day, they got an intraperitoneal infusion of a 15 mg/kg BW lead acetic acid derivation arrangement. To assess the degrees of CK-MB and LDH, blood tests were taken from the rodents' hearts on the twelfth day.

Rat heart tissues were homogenised using 0.1 mM ethylenediamine tetraacetic acid at a concentration of 50 mM and a sodium phosphate buffer with a pH of 7.4. The supernatant was separated following twenty minutes of centrifugation at 1000 g and 4°C.

vi. Measurement of Lactate Dehydrogenase and Creatine Kinase-MB Fraction:

The presence of enzymes like LDH and CK-MB linked to damage to heart cells was examined in the serum. Commercial assay kits were used for all analyses, and the manufacturer's recommendations were followed.

vii. Measurement of MDA:

The quantification of MDA levels in the supernatant of homogenized cardiac tissue was performed using the thiobarbituric acid method. Nanomoles MDA/g tissue was used to express the absorbance at 532 nm.

viii. Measurement of Antioxidant Enzymes:

It was determined that a SOD detection kit was utilised in accordance with the directions provided by the manufacturer in order to ascertain the SOD activity. SOD was measured at a wavelength of 505 nm, which corresponds to the units per milligramme of protein (U/mg). According to the instructions provided by the manufacturer, a GPx detection kit was utilised in order to test the GPx activity. At a wavelength of 340 nm, the GPx was measured by spectrophotometry, and the results were represented as units per milligramme of protein.

ix. Histopathological Examination:

A 10% solution of neutral buffered formalin was used to fix the heart tissue before histological investigation. Hematoxylin and eosin were used for staining.

x. Statistical Analysis:

The results were analysed using one-way ANOVA and were shown as mean±standard deviation (mean±SD). To conduct statistical comparisons between the groups, the LSD test was utilised (SPSS V. 17.0).

IV. Results And Discussion:

i. Characterization of Nanoparticles by Scanning Electron Microscopy and by Dynamic Light Scattering:

The subfields of science and engineering dealing with the design of materials in the nanoscale range are collectively referred to as "nanotechnology". The potential to create nanoparticles from natural sources has been made possible by the relatively recent development of nanotechnology. Compared to a pure natural product, a natural product based on nanoparticles may enhance a medication's bioavailability, solubility, stability, and effectiveness.

Although there are many other polymers that can be used to load herbal extracts into nanoparticles, chitosan in particular is important for the pharmaceutical and medical industries. We produced chitosan nanoparticles by encapsulating the Terminalia arjuna extract using the sodium tripolyphosphate on ionotropic gelation method. There are more advantages to this approach than to the Terminalia arjuna extract.

The surface of the nanoparticles produced by the ionic gelation method exhibited an irregular form and a rough surface morphology, as shown by images captured by scanning electron microscopy (SEM). The production of different nanoparticles in terms of size, form, and composition is now feasible because to recent advancements in nanoparticle technology. Consequently, this has enabled noteworthy advancements to be achieved in the domain of nanomedicine research.

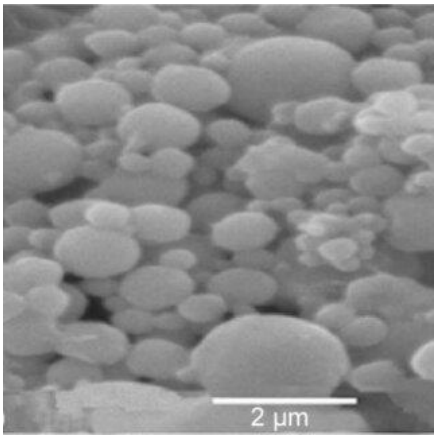

Figure 5: Electron Scanning Microscope the Chitosan-Terminalia arjuna Fruit Image Microparticles be extracted

Nanoparticles have been extensively utilized in the pharmaceutical, medicinal, and therapeutic fields due to their extremely small particle sizes. With sizes smaller than 1000 nm, nanoparticles are highly attractive for pharmaceutical and medical research owing to their unique physicochemical properties, which differ significantly from those of pure materials with the same composition.

The data indicate that administering lead acetate may increase serum levels of LDH and CK-MB, suggesting that lead acetate can induce oxidative stress, damage cardiac cells by producing LDH and CK-MB, and promote lipid peroxidation. Conversely, treatment with Chitosan-Terminalia arjuna extract nanoparticles can prevent oxidative damage and the production of reactive oxygen species (ROS) in cardiac cells, thereby reducing blood levels of CK-MB and LDH.

Table 1: Chitosan-Terminalia arjuna extract nanoparticle size distribution as measured by dynamic light scattering

Size (d. nm)	**0.1**	**1**	**10**	**100**	**-**	**-**	**-**	**1000**	**10000**
	0	**0**	**0**	**0**	**0**	**60**	**0**	**0**	**0**

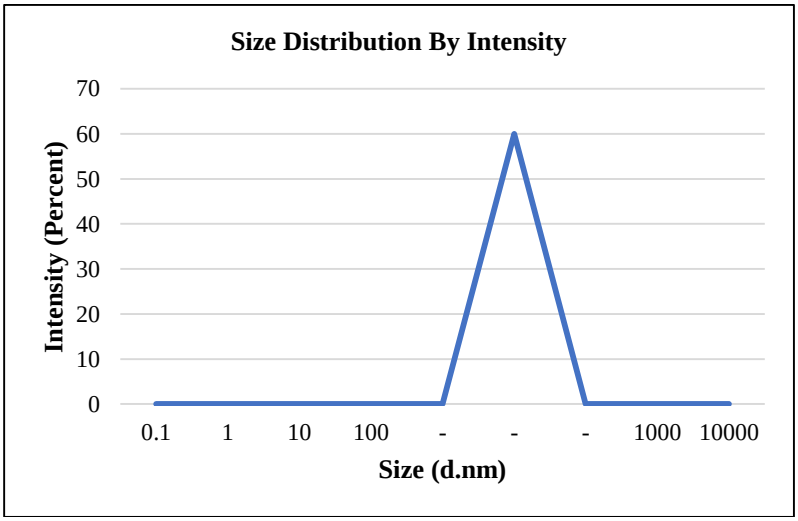

Figure 6:Chitosan-Terminalia arjuna extract nanoparticle size distribution as shown graphically by dynamic light scattering

Table-2: Nanoparticles Derived from Chitosan and Terminalia arjuna Have a Cardioprotective Impact on CK-MB and LDH

Group	CK-MB (IU/L)	LDH (IU/L)
Negative Control	72.8a ± 6.45	98.9a ± 9.56
Positive Control chitosan-T. arjuna	88.7b ± 8.63	269.8b ± 34.74
Chit-T. arjuna Nano 100 mg/kg BW	95.5b ± 8.92	154.4b ± 8.82
Chit-T. arjuna Nano 200 mg/kg BW	88.8b ± 7.48	247.9b ± 9.37
Chit-T. arjuna Nano 400 mg/kg BW	95.5c ± 6.89	232.3c ± 9.78

ii. Chitosan-Terminalia arjuna Reactions Changes in MDA, SOD, and GPx in Heart Tissue Induced by Lead Acetate: An Extract Nanoparticle Analysis:

In the heart tissue of rodents given lead acetic acid derivation, this study analyzed the conceivable defensive capability of nanoparticles containing chitosan and Terminalia arjuna

extricate against oxidative pressure. The table showed the heart tissue MDA results for each gathering. In contrast with the benchmark group, the lead acetic acid derivation gathering's cardiovascular tissue had considerably expanded MDA levels ($P < 0.05$). One of the unsafe outcomes of lead acetic acid derivation on organic frameworks is raised degrees of malondialdehyde (MDA), a consequence of lipid peroxidation and the age of free revolutionaries such hydrogen peroxide, singlet oxygen, and hydro peroxide. The immediate abatement in cancer prevention agent holds, which proposes expanded oxidative pressure in the rodents in the number one spot acetic acid derivation bunch, is another adverse consequence.

Heart tissues treated with Chitosan-Terminalia arjuna extract nanoparticles showed significantly lower MDA levels, indicating a reduction in lipid peroxidation, as compared to the lead acetate group. To be more precise, the cardiac tissue's MDA levels were considerably decreased ($P < 0.05$) by administering 400 mg/kg BW of Chitosan-Terminalia arjuna extract nanoparticles, but dosages of 100 mg/kg and 200 mg/kg BW had no such impact. It is believed that there are two main ways that lead acetate causes oxidative stress and tissue damage: either by directly lowering antioxidant reserves or by causing a rise in reactive oxygen species (ROS). Lead acetate taken orally can enhance lipid peroxidation, affecting cytoplasmic and mitochondrial membranes and worsening oxidative tissue damage.

This study found that nanoparticles made of Chitosan and Terminalia arjuna extract, which have robust antioxidant and free radical scavenging capabilities, were able to lower the lead acetate-induced myocardial damage level in rats. Nanoparticle therapy with Chitosan-Terminalia arjuna extract prevented the increase in MDA levels caused by lead acetate exposure in rats. This proves that the lead acetate's negative effects were mitigated by the antioxidant properties of the nanoparticle made of Chitosan and Terminalia arjuna extract. The antioxidant defence system stops lipid peroxidation, as shown by MDA levels, by lowering oxidative stress and scavenging free radicals that harm the heart. This study's findings suggest that chitosan-terminalia arjuna extract nanoparticles might protect lead acetate-treated hearts from oxidative stress by reducing MDA levels (lipid peroxidation).

Table-3: Chitosan effects on Terminalia arjuna Modifications to Malondialdehyde, Superoxide Dismutase, and Glutathione Peroxidase Induced by Lead Acetate-Extracted Nanoparticles

Group	Means ± Standard Deviation		
	SOD (U/mg tissue)	GPx (U/mg tissue)	MDA (nmol/mg tissue)
Negative Control	8.32 a ± 2.14	1.87 a ± 1.21	58.94a ± 4.26
Positive Control	6.28 b ± 1.83	1.65 b± 1.23	72.25 b ± 7.24
Chit-T. arjuna Nano 100 mg/kg BW	5.53 b ±2.24	1.59 b ± 1.22	79.34 b ±5.23
Chit-T. arjuna Nano 200 mg/kg BW	6.23 b ±1.92	1.60 b ±1.29	73.93 b ±5.40
Chit-T. arjuna Nano 400 mg/kg BW	8.28 c ±2.18	1.80 c ±1.22	65.23 c ±4.53

It has been demonstrated that cellular oxidative stress is correlated with SOD and GPx activity. Lead acetate has been shown in studies to impede antioxidant activity by attaching to and obstructing functional SH groups on a variety of enzymes.

It has been demonstrated that injecting Chitosan-Terminalia arjuna extract nanoparticles lowers lipid peroxidation. Antioxidant defence systems like SOD and GPx, which usually lessen the negative effects of free radicals, have been linked to this impact. Chitosan-Terminalia arjuna extract nanoparticles were administered; they protected the heart with SOD and GPx and decreased CK-MB and LDH activities, possibly stabilizing cell membranes. This stabilization of the membrane may prevent lipid peroxyl radicals from moving and interacting with nearby membrane polyunsaturated fatty acids.

iii. Effects of Chitosan-Terminalia arjuna Extract Nanoparticle on Lead Acetate induce Cardiac Cell Damage:

According to several researches, structural alterations in cardiac tissue in histopathological studies have been linked to heavy metal toxicity, specifically lead acetate. Histological investigations on the negative control in the current study demonstrated that cardiac cells are visible and appear to have a normal architecture of muscular fibres. Rats given lead acetate alone in the positive control group displayed necrosis, or damage to the heart cells. Heart cell number and morphological integrity are maintained in rats administered with Chitosan-Terminalia arjuna extract nanoparticle treatment. The groups administered 400 mg/kg of Chitosan-Terminalia arjuna extract nanoparticle were shown to have minor cardiac damage (necrosis). Chitosan-Terminalia arjuna extract nanoparticles appear to have mitigated the cardiotoxic effects of lead acetate.

V. Conclusion:

This study investigated the cardioprotective effects of nanoparticles made of Chitosan and Terminalia arjuna extract after lead acetate had damaged the hearts of rats. Lead acetate exposure lowered glutathione peroxidase and superoxide dismutase activities while significantly increasing (LDH), (CK-MB), and malondialdehyde (MDA) levels in cardiac tissues. Furthermore, necrosis was confirmed by histological analysis. Nanoparticle therapy with chitosan-Terminalia arjuna extract successfully mitigated these side effects, particularly when administered at a dosage of 400 mg/kg body weight. Its antioxidant and preventive qualities against oxidative stress caused by lead acetate are demonstrated by the considerable reductions in LDH, CK-MB, and MDA levels as well as the increases in SOD and GPx activities. The potential of the nanoparticles to mitigate cardiac damage was further demonstrated by histopathological investigation, which revealed maintained cardiac tissue morphology in the treated groups relative to the lead acetate-only group. These results highlight the potential of Chitosan-Terminalia arjuna extract nanoparticles as a novel therapeutic approach against heavy metal-induced cardiotoxicity, highlighting their function in antioxidant defence and maintenance of cardiac cells.

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