



Therapeutic Potential of Green Synthesized Zinc Oxide Nanoparticles Derived from *Withania coagulans* Extract against High-Fat Diet Associated Hyperlipidemia in Rat Model

Sayed Aman Ullah¹, Faiza Hassan¹, Jahanzaib Khaliq², Ikram Ullah², Shakeel Khan², Sageer Ahmed³, Hina Nawab¹, Aqsa Rashid¹, Amna Arif⁴, Abdul Saboor⁴

¹Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad, Punjab, Pakistan.

²Department of Veterinary Physiology and Biochemistry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Sindh, Pakistan.

³Department of Veterinary Parasitology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Sindh, Pakistan.

⁴Department of Pharmaceutical Chemistry, Govt College University, Faisalabad, Punjab, Pakistan.

ARTICLE INFO

Keywords: Cardiovascular Disease, Dyslipidemia, Hyperlipidemia, *Withania coagulans*, Zinc Oxide Nanoparticles.

Correspondence to: Jahanzaib Khaliq, Department of Veterinary Physiology and Biochemistry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Sindh, Pakistan.

Email: jahanzaibvet@gmail.com

Declaration

Authors' Contribution

All authors equally contributed to the study and approved of the final manuscript.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History

Received: 26-02-2025 Revised: 28-04-2025

Accepted: 08-05-2025 Published: 20-05-2025

ABSTRACT

Background: *Withania coagulans* (Indian rennet, paneer phool, or rishyagandha) an herbal medicinal plant that belongs to the family Solanaceae, common English name "Indian Chees maker" and in Pashto called "Khamazora". **Aim:** the study was conducted to evaluate the anti-hyperlipidemic activity of "*Withania coagulans*". **Methodology:** Total six groups of Wister rats (n=6) and each group with six replicates (r=6) were selected. Each group was labeled as control group, negative control group, Standard group, and Treatment group. Group 1st received normal routine feed with water, group 2nd received a high-fat diet of 30%, groups 3rd, 4th, 5th, and 6th were also fed with a high-fat diet and were treated with the standard anti-hyperlipidemic drug Atorvastatin(2.6mg), low dose (125mg), medium dose (250mg) and high dose (500mg) of *W. coagulans* plant extract using ZnO nanoparticles respectively. Zinc Oxide Nanoparticles (ZnO-NP) were characterized by Zeta size and Zeta potential. Over the course of the 49-day trial, which included 28 days for the induction of hyperlipidemia and 21 days for treatment, the rats were decapitated for the purpose of collecting samples, including blood and various organs, and serological tests were performed to assess the impact of *W. coagulans*. **Results:** A high dose of *W. coagulans* plant extract significantly decreased the elevated level of LDL-C, triglycerides, total cholesterol, ALT, AST, creatinine, and Urea, and significantly increased the low level of HDL-C, RBCs and WBCs count, and SOD. **Conclusion:** *W. coagulans* plant extract utilizing ZnO-NPs had a notable impact on these models of hyperlipidemic-induced rates.

INTRODUCTION

Hyperlipidemia refers to either a high level of low-density lipoprotein cholesterol (LDL-C) more than 130mg/dL, elevated triglycerides > 150mg/dL, or a low level of high-density lipoprotein cholesterol (HDL-C). LDL plays a vital role in the progress of atherosclerosis. Among the various risk factors, dyslipidemia ranked first for the development of cardiovascular diseases (Jacobsen et al., 2024). *Withania coagulans* is also known as Rishyagandha, is a medicinal herb utilized for treating a range of ailments such as indigestion, diabetes mellitus, liver disorders, blood purification, and blood pressure regulation (Gupta et al., 2022). *Withania coagulans* plant is widely distributed across temperate and tropical regions in Asia and is recognized for its diverse pharmaceutical

properties, including anticancer, antilipidemic, and immunosuppressive activities (Tripathi et al., 2005)

Hyperlipidemia continues to be the leading cause of mortality globally, and it is more common in middle- and high-income countries than in low-income ones. Conditions such as obesity, hypertension, blood sugar, and cholesterol levels all have an impact on death rates. These risk factors have been successfully reduced in high-income nations during the last 20 years. High BMI and blood sugar levels, however, are killing more persons in lower- and middle-income groups. Even if fewer people are dying from heart disease, more people are dying overall because of the world's population growth, longer lifespans, and

poor lifestyles that make heart disease prevention more difficult (Barquera et al., 2015).

The fruit of *Withania coagulans*, referred to as Doda Paneer in Hindi and Indian Cheese Maker in English, has been acknowledged for its ethnopharmacological significance in Pakistan's healthcare system. Research has indicated that eating a lot of fat increases the chance of developing diabetes, atherosclerosis, and problems with the macro and microvasculature (Shukla et al., 2014). Obesity and excess weight are closely associated with a number of health problems. Atherosclerosis-related heart disease, diabetes mellitus, and hypertension are among the cardio-metabolic conditions that are significantly influenced by hyperlipidemia. In particular, metabolic dyslipidemia is acknowledged as a crucial link between obesity and a number of cardiovascular diseases (Su et al., 2021).

Dyslipidemia refers to an abnormality in the levels of fat in the bloodstream. Total cholesterol, LDL cholesterol, and triglyceride levels are high, and your HDL cholesterol levels are low. It is a major risk factor for cerebrovascular and cardiovascular events. To lower the risk of myocardial infarction and stroke, dyslipidemia must be prevented and managed. Over 15% of deaths each year are caused by heart attacks, a frequent and fatal cardiac condition that primarily affects males. About 7.4 million people died from coronary heart disease (CHD) in 2015; by 2030, that number is predicted to rise to 23.6 million. Stroke is the second leading cause of mortality and a major contributor to chronic health issues. It accounts for 11.13% of all fatalities globally. Strokes affect 0.2% of the population annually, with a high death rate in the first month and over half of survivors experiencing severe impairment (Alloubani et al., 2021).

Dyslipidemia is considered the main cause of cardiovascular disease and stroke. Epidemiological studies show that people with healthier lifestyles face fewer chances of cardiovascular diseases. Increased cholesterol level is the root cause of atherosclerotic cardiovascular disease. According to Kopin and Lowenstein (2017), atherosclerotic cardiovascular disease (ASCVD) is the primary cause of death and morbidity in both types of diabetes. Dyslipidemia can be inherited or associated with diabetes mellitus. The most prevalent cause of cardiovascular problems is hypercholesterolemia. For passing in 1990, 2007, and 2019, high LDL is the 15th, 11th, and 8th most dangerous driving hazard, respectively. Obesity and atherogenic dyslipidemia are closely related. Quantitative and qualitative alterations in plasma lipoproteins are hallmarks of atherogenic dyslipidemia, a lipid disorder. It is more prevalent in women and is closely linked to obesity. It is also possibly the most dangerous factor for reproductive issues, pregnancy-related metabolic illnesses, and cardio-metabolic infections in later life (Vekic et al., 2023).

Studies have shown that nanotechnology can be used to cure more than half of CVDs. Examining current developments in cardiovascular medication carriers based on nanoparticles is the main goal of this study. Furthermore, this study seeks to describe the difficulties

with traditional treatment approaches in contrast to nanomedicine's potential to cure CVDs (Pala et al., 2020).

Herbal drugs derived from medicinal plants have the potential to revolutionize the healthcare industry by providing a natural and effective alternative to synthetic drugs. However, to ensure their best usage in treating human illnesses, more study and standardization are necessary, since worries about the safety and effectiveness of herbal medicines persist (Rasool et al., 2020). Natural herbs have the ability to solve the problem of drug resistance in microorganisms, which is why their use as main components in the creation of innovative medicines is continuously increasing. Due to the growing need for medicinal plants in both developed and developing countries, research on these plants is becoming increasingly important on a global scale. But greater attention must be paid to the preservation of medicinal plants and the assessment of bioactivity-safety (Soetan et al., 2009).

Nanoparticles' extraordinary power and functional capabilities make them essential to the fields of nanoscience and nanotechnology. These particles are usually made by physical or chemical means, but their potential toxicity restricts their application. Various environmentally benign and less harmful green synthesis methods, such as using plants, microbes, and algae, have been investigated in response to this constraint. Among these green synthesis techniques, the use of medicinal plants is especially important as it presents a potential remedy for the problems of toxicity and bacteria multidrug resistance that are present in the existing treatment strategies. In light of the general interest in metal nanoparticles, this study focuses on the environmentally friendly and sustainable production of metallic nanoparticles utilizing medicinal herbs (Chandra et al., 2020)

Currently, heart disease is the leading cause of mortality globally. Atherosclerosis starts during childhood and then gives way during adolescence to cardiovascular diseases later in life after reaching young adulthood. Several cardiovascular risk factors can be identified in childhood and adolescence. However, hyperlipidemia has emerged as the most common factor linked to the central development of ASCVD as a result of the worldwide obesity pandemic. Therefore, screening for hyperlipidemia is crucial in order to identify children who are at high risk of developing these illnesses; these individuals should receive more thorough research and treatment. In order to reduce the chance of developing ASCVD in the future, treatment should begin as soon as feasible. Numerous factors contribute to the fact that heart illnesses are the leading cause of mortality worldwide. Plaque buildup in the blood arteries causes the atherosclerotic condition; this pathogenic process begins in adolescence, continues throughout life, and ultimately leads to heart disease (Mainieri et al., 2023).

An aging population and declining infections are the primary factors responsible for the global increase in cardiovascular disease, with three trends in developing countries being at the forefront: (1) better nutritional intake and (2) lower mortality rates (3) the sharp increase

in the smoking rate, which already increases cardiovascular vascular disease and also causes an increase in smoking-related cancers if the use lasts longer (Hennekens et al., 2000).

CVDs remain the predominant reason for mortality in Asia, accounting for 58% of the total CVD deaths worldwide in 2019. In Asia, CVD caused 10.8 million deaths that year, accounting for 35% of all deaths, with 39% of those deaths happening in those under 70, which is higher than the rates in the US, Europe, and the rest of the world. Eighty-seven percent of the fatalities from CVD were caused by ischemic heart disease and stroke. Between 1990 and 2019, the number of fatalities in Asia from CVD increased from 5.6 million to 10.8 million, from 23% to 35% of total deaths in the area. Demographic changes caused the crude death rate to increase, while the age-standardized mortality rate decreased in response. The development of successful public health initiatives depends on these epidemiological trends (Zhao et al., 2021).

Heart disease is the leading cause of mortality globally, and its prevalence has also risen in low- and middle-income countries. Although effective preventative strategies are available, each demographic requires a customized strategy. The efficacy of public health efforts depends on the gathering of local data on atherosclerotic disorders. Thorough monitoring and testing can help reduce the effects of heart disease. This can aid in the prevention and treatment of issues including heart attacks and strokes. (Franco et al., 2011)

Cardiovascular diseases (CVDs) pose a significant health challenge globally, with a growing prevalence and being a primary cause of illness and death. Herbal remedies are becoming more popular as alternative options for treating different conditions, including CVDs, due to their cost-effectiveness and perceived safety in comparison to modern medications. Despite the increasing use of medicinal herbs, there is a lack of comprehensive testing on their safety, highlighting the need for greater public awareness regarding potential risks, side effects, and interactions with conventional drugs. Research conducted in laboratories indicates that medicinal herbs could offer therapeutic benefits for CVDs by influencing various risk factors. As a result, there is a push to move these laboratory findings into clinical settings to effectively incorporate herbal treatments into the management of cardiovascular diseases (Shaito et al., 2022). CVD is now considered a significant threat to the well-being of humans. Even though there are different types of heart disease medications available, they are not always effective because they don't dissolve well in water, don't work well in the body, don't target the problem area, and can stop working overtime. Nano-drugs delivery systems were developed by us to aid in the treatment of heart diseases. This new method is proving to be extremely beneficial in addressing the issues we previously discussed. However, there are also problems with NDDSs that need to be fixed, such as their toxicity (Deng et al., 2020).

The risk of atherosclerosis and subsequent cardiovascular diseases is high when hyperlipidemia is

present. An abnormal elevation in blood cholesterol, particularly elevated levels of LDL and triglycerides, is the hallmark of hyperlipidemia, which is influenced by genetic and environmental factors. Current therapy consists of lifestyle modification and pharmacological interventions, mostly consisting of statins. As the field of managing hyperlipidemia continues to evolve, it is crucial to continue exploring existing treatments as well as new and potential candidates, which could lead to improved treatment strategies in the future (Abbasi et al., 2024).

Atherosclerosis is a chronic disease caused by inflammation of the artery wall and an alteration in blood lipids. It's the biggest killer in industrialized countries. Medicinal herbs have lipid-lowering, antioxidant, and antibacterial qualities that can prevent atherosclerosis. These substances are utilized extensively. These plants have been found to have less adverse effects and to be useful in the prevention and treatment of atherosclerosis (Kirichenko et al., 2020).

Withania coagulans is a medicated plant known as Cheesemaker or vegetable Rennet and traded as Paneer. It belongs to the family of Solanaceae. The medicinal plant is very famous for its significant antihyperglycemic and antihyperlipidemic properties. Principal extraction modes applied to this plant are alcoholic, methanolic, ethanolic, and aqueous extractions (Bandawane and Juvekar, 2021).

MATERIALS AND METHODS

Research Area

The study was carried out at the University of Agriculture, Faisalabad, in the lab of the Department of Pharmacology and the relevant animal house.

Bioethics Permission

After receiving approval from the University of Agriculture Faisalabad's Bioethics committee, all experimental research trials were conducted. In addition to a printed copy of the study proposal being sent to the committee, the research specifics and goal were presented to the committee.

Experimental Animals

For the study, 36 healthy Wistar albino rats (120–130g, 4 weeks old) were used over a 48-day period (Table 1). Hyperlipidemia was induced by feeding them a high-fat diet made from 30% rat feed and 70% cooking ghee for 30 days. The rats' body weight was recorded weekly. Initially, the rats showed normal growth, which slowed after starting the high-fat diet.

Table 1

Experimental Design

Group No	Group Name	Number of Animals	Treatments
1)	Control group	6	Routine feed and normal saline
2)	Negative control group	6	High Fat Diet 30% induced hyperlipidemia untreated
3)	Standard Group	6	High Fat Diet 30% induced hyperlipidemia will be treated with standard drug Atorvastatin (2.6mg/kg)
4)	Treatment group	6	HFD 30% induced hyperlipidemia will be

			treated with a low dose of plant extract using zinc oxide nanoparticle (125mg/kg)
5)	Treatment group	6	HFD 30% induced hyperlipidemia will be treated with a Medium dose of plant extract using zinc oxide nanoparticles (250mg/kg)
6)	Treatment group	6	HFD 30% induced hyperlipidemia will be treated with a high dose of plant extract using zinc oxide nanoparticle (500mg/kg)

Induction of Hyperlipidemia

Figure 1(a) & (b)



Herbal Plant Collection

The plants were gathered from my hometown of Zhob, which is in the northern region of Pakistan's Baluchistan province, in order to assess *Withania coagulans*' anti-hyperlipidemic potential. To get rid of the dust and other debris, the leaves, fruits, and roots of the plant were all gathered and carefully cleaned with tap water.

Herbal Plant Identification

I submitted a sample of a plant's seeds and leaves to the Department of Botany and Agriculture at the University of Agriculture Faisalabad (Figure 2) for identification and analysis. Experts identified the plant and found several bioactive compounds such as flavonoids, terpenoids, and alkaloids with various pharmacological benefits. They also conducted a qualitative and quantitative analysis to determine the levels of total flavonoid content (TFC) and total phenolic content (TPC).

Figure 2

Herbal plant extract



Zinc Oxide Nanoparticles preparation

Nanoparticles of zinc oxide were employed in the study. The recommended process is followed in the preparation of these particles. The following substances are utilized to create nanoparticles: 1. Metal nanoparticles (copper, iron, zinc and silver). 2. Iron oxide, aluminum oxide, and zinc oxide are examples of metals and non-metals oxides. 3. Semiconductor, including zinc nitrate and zinc sulfate. 4. Carbon (C) nanoparticles

Low dose

A low dose of 125mg/kg of zinc oxide nanoparticles containing the plant extract was made using a weighing balance, spatula, Falcon tube, and distilled water.

Medium dose

A medium dose of 250mg/kg of zinc oxide nanoparticles containing the plant extract was made using a weighing balance, spatula, Falcon tube, and distilled water.

High Dose

A high dose of 500mg/kg of zinc oxide nanoparticles containing the plant extract was made using a weighing balance, spatula, Falcon tube, and distilled water.

Standard drug

Atorvastatin which is a lipid-lowering drug was used in comparison with prepared doses (Low dose, medium dose, and high dose) of medicinal plants. The dose rate of atorvastatin was 2.6 mg

Decapitation

As the research duration ended, all the experimental rats were ethically decapitated for tissue sampling. Before sample collection, all the bio safety protocols were followed. Following materials are used such for tissue sampling such as, Tissue preservative formalin, Icebox, Scissors and surgical blades, Petri dish, Surgical gloves and face mask, Blood collection tubes, Apendrops, Triazoles, Polythene bags, and Alcohol (Figure 3).

Figure 3

Decapitation of rats



Blood and Organs Sampling

Procedure

Initially, all standard self-protection protocols, including wearing a gown, gloves, and face mask, were followed. Alcohol was used to anesthetize the rats before cutting the jugular veins with a sterile blade for blood collection in EDTA tubes. The samples were labeled with rat numbers and sent to the CMS department at the University of Agriculture, Faisalabad, for hematological analysis using

an automated blood analyzer. Additionally, the aorta and adipose tissues were extracted for further examination (Figure 4a, 4b, and 5).

Figure 4(a) & 4(b)

Sampling of aorta from heart



Figure 5

Adipose tissue collections



Hematological Analysis

In order to get tissue and blood samples, the rats were decapitated. After that, the blood sample was centrifuged for 15 minutes at 1500 revolutions per minute, which is how plasma and serum are separated. Then, these were kept at -20°C.

Serum Analysis

In serum analysis, RBCs and WBC count were determined. In the case of hyperlipidemia, oxidative stress is caused due to which the RBC counts lower than normal value. In contrast, the WBCs level is normal in healthy rats while increases in hyperlipidemic rats

Complete Blood Count (CBC)

The pathology diagnostic lab at the University of Agriculture Faisalabad processed the collected blood samples. All of the blood's components were extracted from an automatically created report. The concentrations of hemoglobin, white blood cells, and red blood cells were compared to normal values

Liver Function Tests (LFTs)

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) are two enzyme reaction assays that may be used to test liver function. Measurements were made of these liver-produced enzymes that control bodily processes. Normal values and the measured values of these enzymes were contrasted.

Renal Function Tests (RFTs)

1. Urea

The Urease-GLDH method is used for renal function tests.

Creatinine determination

The most commonly used method is the **Jaffe reaction**.

2. Lipid Profile

Triglycerides (TG)

Triglycerides were quantified using the commercially accessible QCA® kit from Spain.

3. Total Cholesterol (TC)

The measurement of total cholesterol levels was determined by using a commercially accessible QCA® kit in the country of Spain.

4. High-density lipoprotein (HDL-C) Cholesterol

High-density lipoprotein (HDL-C) Cholesterol was determined through a commercially available QCA® kit, in Spain.

5. Low-density lipoprotein (LDL-C) Cholesterol

The colorimetric technique was utilized to measure the concentration of serum total cholesterol in vitro by employing a commercially accessible QCA® kit from Spain.

Anti-oxidant Activity

Superoxide dismutase (SOD)

The enzymatic function of SOD in both serum and liver tissue was assessed using the protocol outlined by Gianopolitis and Reis.

Histopathological Examination

Following the procedure, tissue samples were rinsed with 0.9% saline and preserved in 10% formalin. Histological analysis was conducted using the method of Bancroft and Gamble (2008), involving paraffin wax impregnation, hematoxylin and eosin staining, and examination under a light microscope. Phosphate buffer formalin was used for sample preservation during the analysis.

Statistical Analysis

Statistical analysis involved the utilization of one-way and two-way ANOVA tables within the framework of completely randomized design (CRD) and Graph Pad Prism software.

RESULTS

Phytochemical Analysis of *Withania Coagulans*

Qualitative analysis

Through qualitative analysis of *W. coagulans*, different bioactive constituents were identified. These bioactive constituents possess different pharmacological properties such as anti-hyperlipidemic, anti-hyperglycemic, anti-oxidant, anti-inflammatory, and anti-convulsant (Table 2).

Table 2

Phytochemical analysis of Withania coagulans

Serial No.	Phytoconstituent	Present	Absent
1	Withanolides	+	
2	Alkaloids		-
3	Steroidal Lactones		-
4	Saponins	+	
5	Flavonoids	+	
6	Glycosides	+	
7	Tannins	+	

Hematological parameters

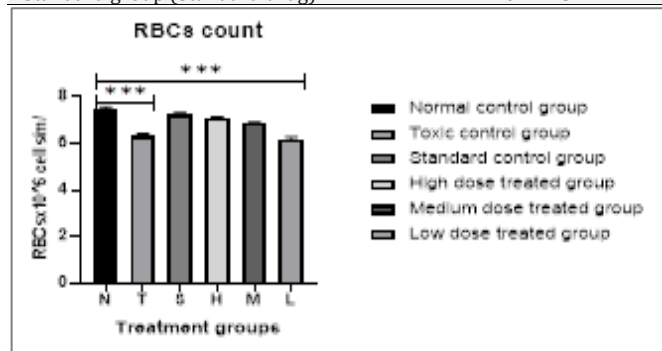
Red blood cell count

The table shows a significant increase in RBC's count in all treated groups. In the case of hyperlipidemia, oxidative stress may develop and it causes hemolysis of RBCs and hence decreased in RBCs count (Table 3).

Table 3

The effect of Withania coagulans extract on red blood cell count (Mean ± SE) in hyperlipidemic-induced and treated groups.

Groups	Mean ± SE (P<0.05)
Normal Control group	7.50± 1.15
Toxic Control group	6.40± 1.02
Treatment group 1 (Low dose)	6.15±0.849
Treatment group 2 (Medium dose)	6.90± 1.00
Treatment group 3 (High dose)	7.00±1.25
Standard group (Standard drug)	7.20± 1.25



Significant***= A graphic depiction reveals that the body's RBC count is initially high. The RBC level decreases when hyperlipidemia is introduced. The high dose of the plant extract and the standard medication had nearly identical levels of effectiveness.

Effect of *W. coagulans* plant extract on White Blood Cells (WBCs) count

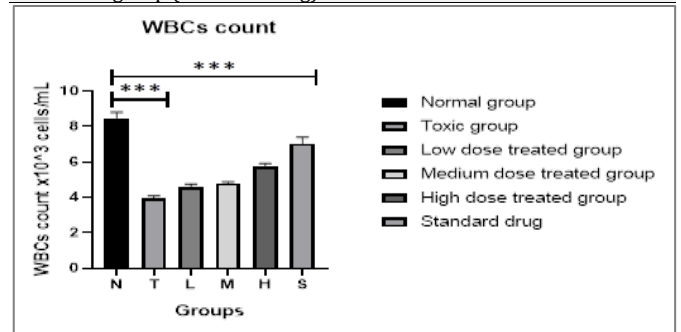
The WBC counts returned to normal after the *Withania coagulans* plant extract was administered to these toxic or hyperlipidemic rats. Thus, this finding suggests that using

Withania coagulans plant extract as an anti-hyperlipidemic treatment significantly increased the WBC count (Table 4).

Table 4

The effect of Withania coagulans extract on White blood cell count (Mean ± SE) in hyperlipidemic-induced and treated groups.

Groups	Mean ± SE (P<0.05)
Normal Control group	8± 1.15
Toxic Control group	5.40± 1.02
Treatment group 1 (Low dose)	2.50±0.849
Treatment group 2 (Medium dose)	3.50± 1.00
Treatment group 3 (High dose)	5.50±1.25-
Standard group (Standard drug)	6.20± 1.25



Significant***= The WBC count graphic indicates that the rats' WBC levels were initially high prior to the introduction of hyperlipidemia. When hyperlipidemia was added, the toxic (T) group's WBC level significantly dropped. The levels of WBC in the treatment groups of lower dosage, medium dose, high dose, and standard medication increased considerably when these hyperlipidemic rats were given extract of *W. coagulans*.

Lipid Profile Tests

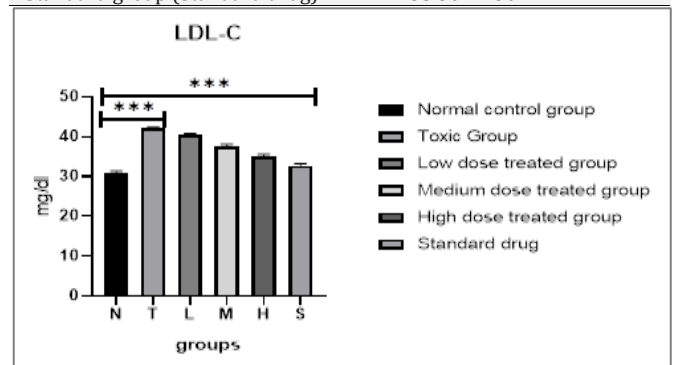
Effect of *W. coagulans* plant extract on LDL-C

Table 5 show that *Withania coagulans* is a plant extract that has been shown to reduce high levels of bad cholesterol (LDL-C), raise good cholesterol (HDL-C), and lower triglycerides. Overall, the plant extract has significantly reduced blood fat levels after producing elevated blood fat (Table 5).

Table 5

The effect of Withania coagulans extract on LDL-C (Mean ± SE) in hyperlipidemic-induced and treated groups.

Groups	Mean ± SE (P<0.05)
Normal Control group	31± 1.20
Toxic Control group	42.50± 1.10
Treatment group 1 (Low dose)	41.50±0.849
Treatment group 2 (Medium dose)	38.40± 1.00
Treatment group 3 (High dose)	35.60±1.25
Standard group (Standard drug)	33.50± 1.50



Significant***= LDL-C graphic depiction reveals that the normal (N) group has a low level of LDL-C. As in the toxic (T) group, the level of LDL-C rose considerably following the creation of hyperlipidemia. When *W. coagulans* extract was administered to these hyperlipidemic rats, the treatment groups' LDL-C levels dramatically dropped. Similar effects were observed in the effectiveness of common medication atorvastatin and large dosages of plant extract.

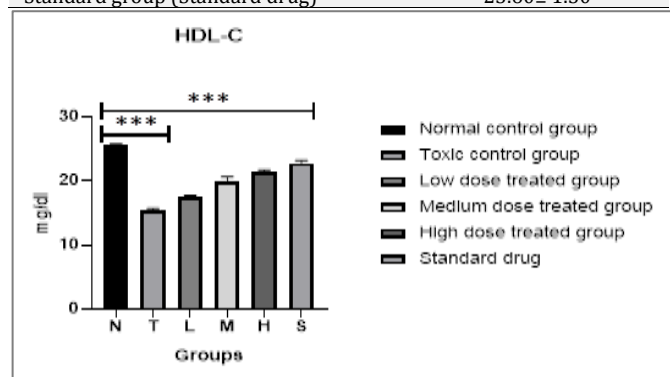
Effect of *W. coagulans* plant extract on HDL-C

Good cholesterol HDL-C is typically high in healthy rats, but its value drops when hyperlipidemia is created. Thus, when hyperlipidemic rats are given a *W. coagulans* extract, their values return to normal. This suggests that *W. coagulans* plays a crucial part in preserving the body's HDL-C level (Table 6).

Table 6

The effect of Withania coagulans extract on HDL-C (Mean ± SE) in hyperlipidemic-induced and treated groups.

Groups	Mean ± SE (P<0.05)
Normal Control group	25.80± 1.20
Toxic group	15.80± 1.10
Treatment group 1 (Low dose)	17.50±0.849
Treatment group 2 (Medium dose)	19.50± 1.00
Treatment group 3 (High dose)	21.40±1.25
Standard group (Standard drug)	23.60± 1.50



Significant***= The HDL-C graphic indicates that the normal (N) group has a high HDL-C level. Similar to the toxic (T) group, the level of HDL-C dramatically dropped when hyperlipidemia was induced. When *W. coagulans* extract was administered to these hyperlipidemic rats, the treatment groups' HDL-C levels dramatically rose.

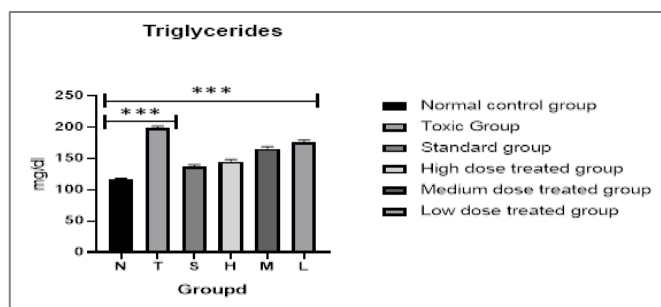
Triglycerides

Triglyceride levels are significantly elevated in hyperlipidemic rats. After treatment of these induced hyperlipidemic rats with plant extract of *W. coagulans*, there was a significant decrease in the level of triglycerides (Table 7).

Table 7

The effect of Withania coagulans extract on Triglycerides (Mean ± SE) in hyperlipidemic-induced and treated groups.

Groups	Mean ± SE (P<0.05)
Normal Control group	116± 1.20
Toxic group	202± 1.10
Treatment group 1 (Low dose)	170±0.849
Treatment group 2 (Medium dose)	165± 1.00
Treatment group 3 (High dose)	145±1.25
Standard group (Standard drug)	130± 1.50



Significant***= Graphical representation shows that initially, the level of triglycerides in the body was normal. When hyperlipidemia is introduced, the level of triglycerides was increased.

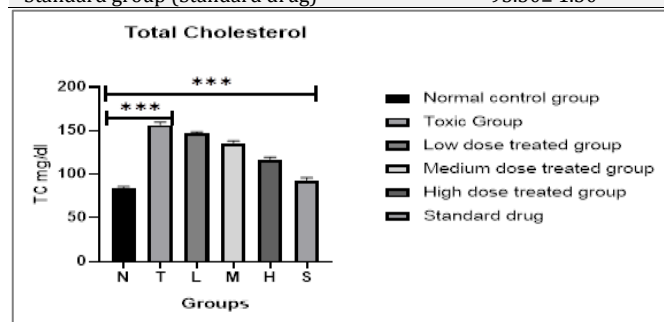
Total Cholesterol

The normal rats had normal cholesterol levels, but the rats with high cholesterol had much higher levels. After giving the rats with high levels of fats in their blood a treatment with *W. Extract* from coagulants plant reduces total cholesterol level to a normal range (Table 8).

Table 8

The effect of Withania coagulans extract on Total Cholesterol (Mean ± SE) in hyperlipidemic-induced and treated groups.

Groups	Mean ± SE (P<0.05)
Normal Control group	80.83± 1.20
Toxic group	156.50± 1.10
Treatment group 1 (Low dose)	145.50±0.849
Treatment group 2 (Medium dose)	130± 1.00
Treatment group 3 (High dose)	110.50±1.25
Standard group (Standard drug)	95.50± 1.50



Significant***= Graphical representation shows that initially, the level of total cholesterol level was very low in contro group. When hyperlipidemia is introduced, the level of cholesterol level was increased.

Liver Function Tests

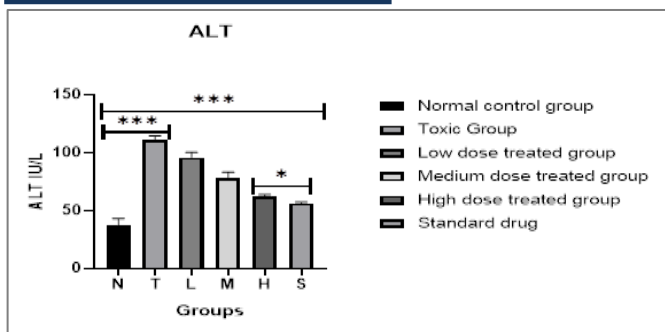
Alanine Aminotransferase (ALT)

After hyperlipidemic induction, a significant increase in ALT level was seen while after treatment with plant extract, there was a significant decrease in ALT level (Table 9).

Table 9

The effect of Withania coagulans extract on Alanine Aminotransferase (Mean ± SE) in hyperlipidemic-induced and treated groups.

Groups	Mean ± SE (P<0.05)
Normal Control group	30-50 IU/L± 1.20
Toxic group	110.50± 1.10
Treatment group 1 (Low dose)	95.60±0.849
Treatment group 2 (Medium dose)	70.50±1.00
Treatment group 3 (High dose)	60.60±1.25
Standard group (Standard drug)	55.50± 1.50



Significant***= Graphical representation shows that initially, the level of ALT in the body was low. When hyperlipidemia is introduced, the level of ALT was increased.

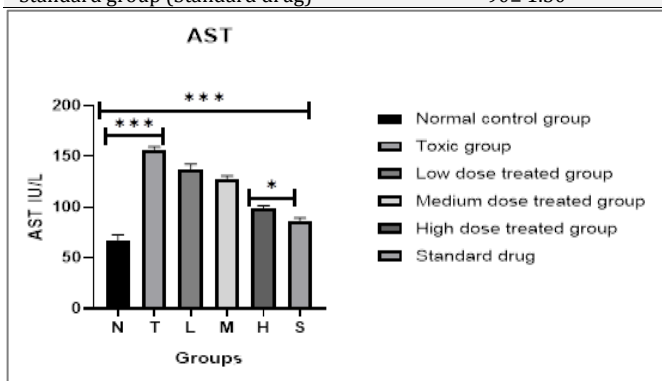
Aspartate Aminotransferase (AST)

The following table depicts that there is an increased level of AST in all hyperlipidemic experimental groups. After treatment with plant extract of *W. coagulans*, there is a significant decrease in AST level (Table 10).

Table 10

The effect of Withania coagulans extract on Aspartate Aminotransferase (Mean ± SE) in hyperlipidemic-induced and treated groups.

Groups	Mean ± SE (P<0.05)
Normal Control group	60-80± 1.20
Toxic group	150-160± 1.10
Treatment group 1 (Low dose)	130-140±0.849
Treatment group 2 (Medium dose)	120±1.00
Treatment group 3 (High dose)	100±1.25
Standard group (Standard drug)	90± 1.50



Significant***= Graphical representation shows that initially, the level of AST in the body was low. When hyperlipidemia is introduced, the level of AST was increased.

Renal Function Tests (RFTs).

Urea

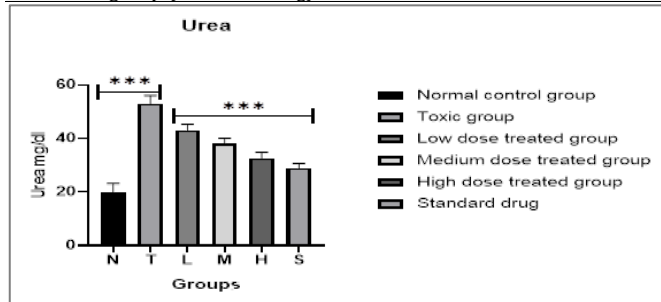
The level of urea was measured in renal function testing. The urea level rises following the development of hyperlipidemia. The level of this kidney function biomarker has been considerably restored by the plant extract of *W. coagulans* (Table 11).

Table 11

The effect of Withania coagulans extract on Urea (Mean ± SE) in hyperlipidemic-induced and treated groups.

Groups	Mean ± SE (P<0.05)
Normal Control group	15-25± 1.60
Toxic group	50mg± 1.50
Treatment group 1 (Low dose)	40±0.589

Treatment group 2 (Medium dose)	35±1.00
Treatment group 3 (High dose)	30±1.75
Standard group (Standard drug)	28± 1.80



Significant***= It shows that there is a significant increase in the level of Urea after induction of hyperlipidemia. On the other hand (Right side of the graph) there is a significant decrease in the level of urea when treatment is given.

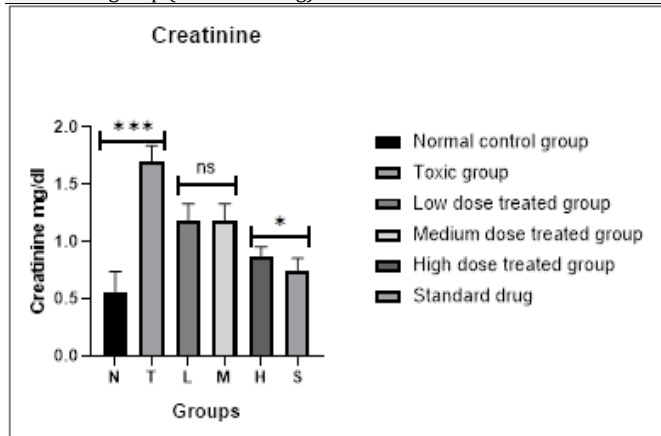
Creatinine Level

Creatinine levels were monitored in renal function testing. Creatinine levels rise with the development of hyperlipidemia. The amount of this renal function biomarker has been considerably restored by the plant extract of *W. coagulans* (Table 12).

Table 12

The effect of Withania coagulans extract on Creatinine Level (Mean ± SE) in hyperlipidemic-induced and treated groups.

Groups	Mean ± SE (P<0.05)
Normal Control group	0.4-0.8± 1.60
Toxic group	1.5± 1.50
Treatment group 1 (Low dose)	1.3±0.589
Treatment group 2 (Medium dose)	1.2±1.00
Treatment group 3 (High dose)	1.05±1.75
Standard group (Standard drug)	0.9± 1.80



Significant***= There is a significant increase in the level of creatinine after induction of hyperlipidemia. There is a Non-Significant relationship between the low and medium doses. On the right side of the graph, a single * indicates a minute significant relationship between a high dose of plant extract and the standard dose.

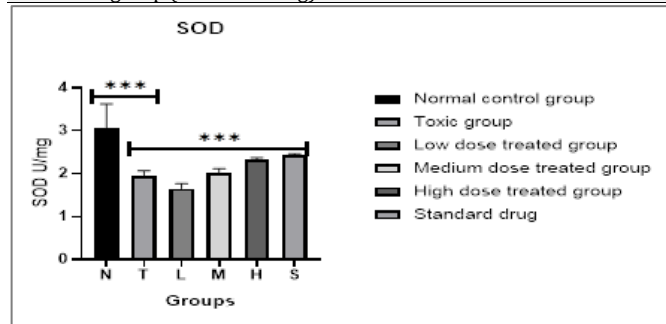
Superoxide Dismutase (SOD), an anti-Oxidant

Superoxide dismutase, an antioxidant, is often present in high concentrations in the body to protect it from stress. Because of oxidative stress, SOD levels fall in hyperlipidemic situations. The SOD level in hyperlipidemic rats was considerably raised to the normal range after they were given an extract of *W. coagulans* (Table 13).

Table 13

The effect of *Withania coagulans* extract on Superoxide Dismutase (SOD) (Mean ± SE) in hyperlipidemic-induced and treated groups.

Groups	Mean ± SE (P<0.05)
Normal Control group	2.5-4.0± 1.60
Toxic group	1.90± 1.50
Treatment group 1 (Low dose)	1.95±0.589
Treatment group 2 (Medium dose)	2.00±1.00
Treatment group 3 (High dose)	2.4±1.75
Standard group (Standard drug)	2.5± 1.80



Significant***= there is a significant increase in the level of SOD when hyperlipidemic rats were treated with plant extract.

Histology of organ samples

Pathophysiology of Cardiovascular Disease

The primary cause of heart disease and stroke is hyperlipidemia. The first step in the development of CVDs is the accumulation of LDL-C in the innermost endothelial layer of any tubular organ, which leads to atherosclerotic formation. The engulfing process of macrophages results in the creation of foam-like cells. This study demonstrates that the plant extract *Withania coagulans* prevents the production of plaque or atherosclerosis, thereby having an antihyperlipidemic effect. The physiology of the liver, kidney, gut, and fat tissues showed a notable improvement.

Liver tissues histology

Fatty liver is the term used to describe the accumulation of fat on the liver in cases of hyperlipidemia. An imbalance in liver function is caused by a fatty liver. Several metabolic hormones, including bilirubin, ALT, ALP, and AST, are produced by the liver. These liver indicators are elevated in cases of hyperlipidemia. These hormones significantly improve following treatment with *W. coagulans* plant extract, demonstrating the impact of the extract on liver tissues. (Figure 6a, 6b, 6c, 6d, 6e, 6f)

Figure 6a

Normal Liver tissues

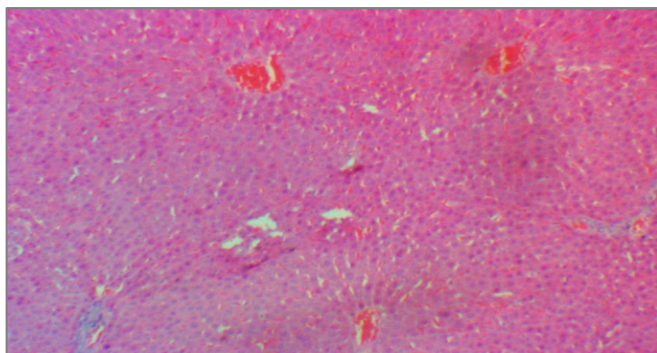
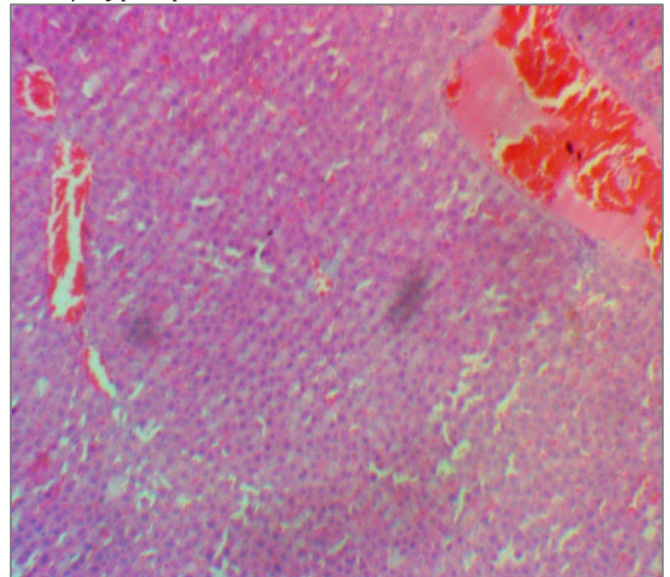


Figure 6b

Toxic / Hyperlipidemic liver tissues



Treatment Group Liver Tissues

Figure 6c L.T,

Low dose of plant extract

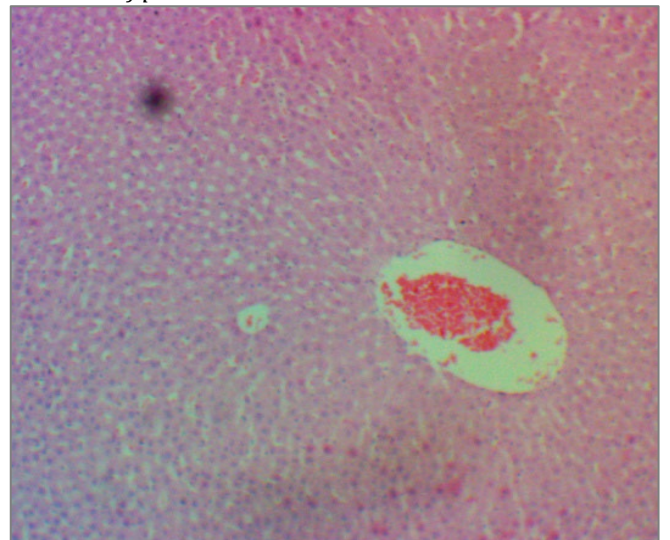


Figure 6d L.T,

Medium dose of plant extract

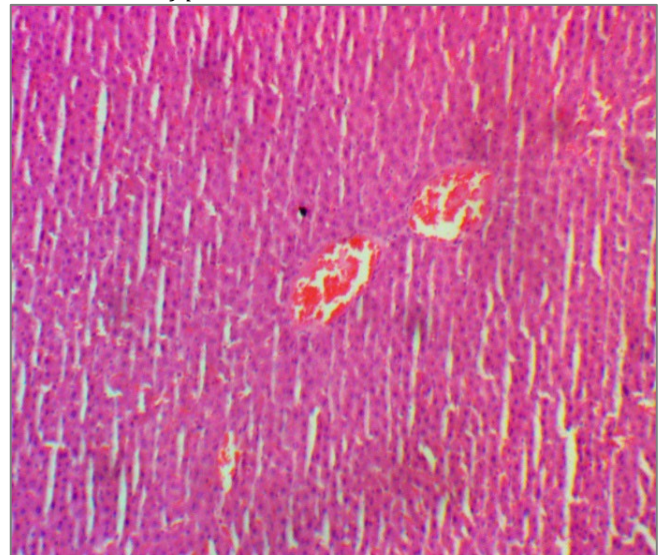
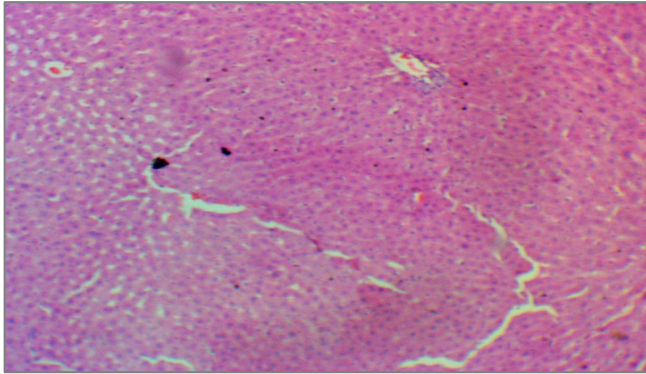


Figure 6e L.T,
High dose of plant extract



Figure 6f L.T,
Standard drug (Atorvastatin)



Histopathology of Adipose Tissues

Figure 7a
Normal Adipose tissue

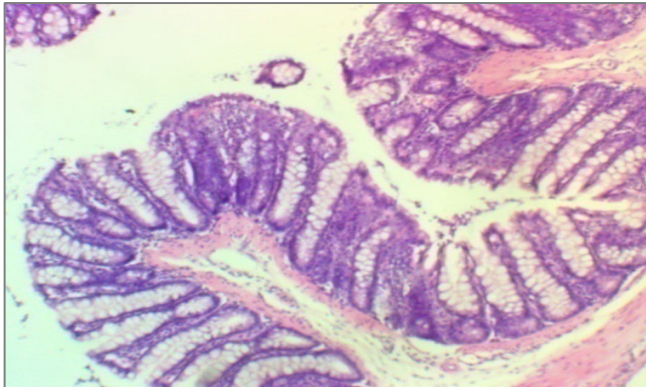
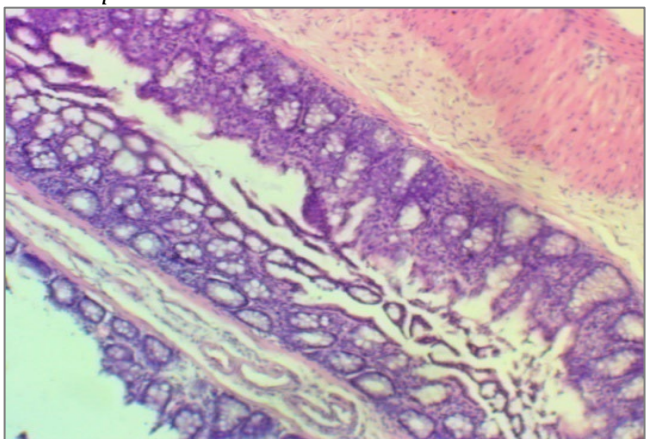


Figure 7b
Toxic adipose tissue



Treatment groups adipose tissue histology

Figure 7c
Low dose treated adipose tissue

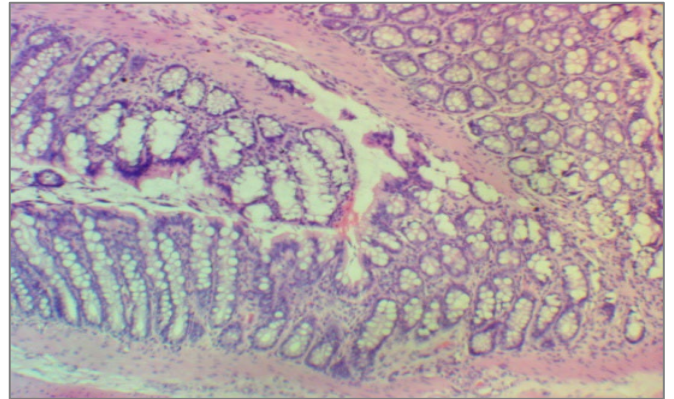


Figure 7d
Medium dose treated adipose tissue

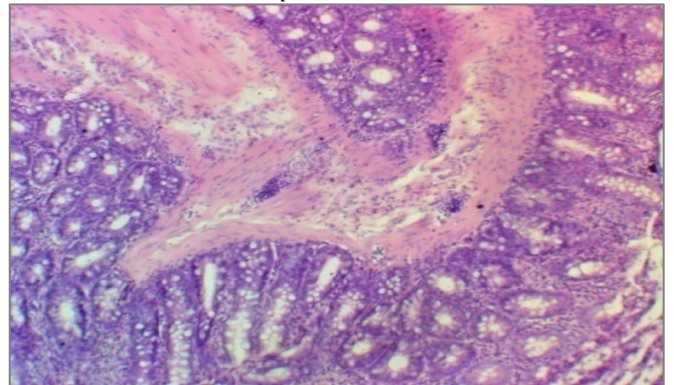


Figure 7e
High dose treated adipose tissue

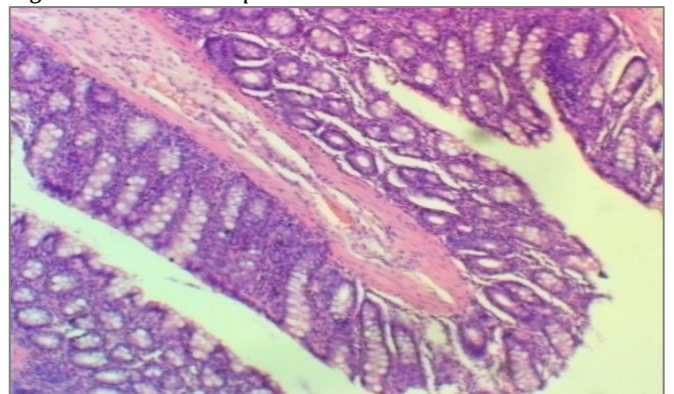
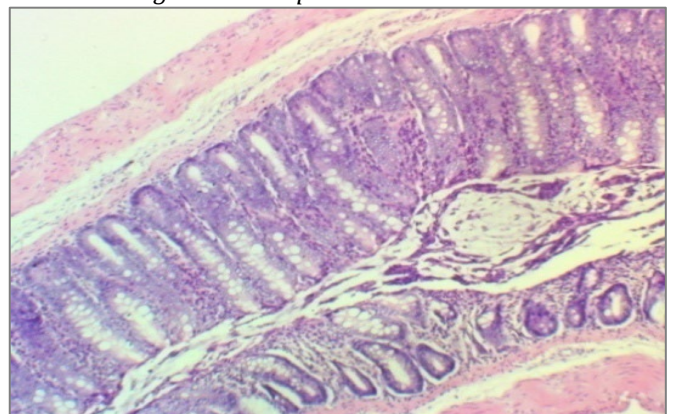


Figure 7f
Standard drug treated adipose tissue



DISCUSSION

The present study was carried out on the medical uses of the herbal plant *Withania coagulans* to treat disease associated with consuming of high fat diet. According to our research RBCs and WBCs count are decreased up to 6.40 ± 1.02 , 5.40 ± 1.02 respectively, in hyperlipidic induced rats, while increased again in RBCs and WBCs count up to normalized range when treated with *Withania coagulans* extract. Same results were concluded by (Abdelhalim 2010; Khan et al., 2024) where the RBCs count are decreased and unlike WBCs and platelets count are increased in hyperlipidic induced rats and physiologically stressed rabbits by introducing B-Oligosaccharide and probiotic mixture. However, RBCs value is normally high in adult female of animals and its values will be decreased in diseases condition (Arif et al., 2024).

Our results show that there is increased in LDL-C value upto 42.50 ± 1.10 in hyperlipidic induced rats and decreased again in treated groups, same results were presented by (Abdelhalim 2010). Same The fat value was also decreased in rabbits meat feeded with prebiotic supplementation (Ahmed et al., 2024).

Furthermore, in present study good cholesterol HDL-C value 15.80 ± 1.10 was decreased in hyperlipidic induced rats and increased in treatment groups, unlike there was increased in HDL-C level presented by (Yang et al., 2025), while there was no significant difference in HDL-C in hyperlipidic induced rats (Ogunola et al., 2010).

Similarly in present study there was significant increase of triglycerides and total cholesterol levels 202 ± 1.10 , 156.50 ± 1.10 respectively in hyperlipidic induced rate and again attained the normal ranges when treated with mentioned herbal plant. This data is matched with other researcher (Laaboudi et al., 2025)

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) enzymes are used to check the liver function in diseases condition; its values are irregular in normal or diseases condition. Our results show that there was normal values of both enzymes in control group, while there were significant increased in both enzymes value 110.50 ± 1.10 , $150-160 \pm 1.10$ respectively in hyperlipidic induced rats, same results are concluded by (Yoshikiyo et al., 2025). Sometimes these enzymes value increase or decrease due to some other factors, not due to the abnormal function of liver (Shazinosh et al., 2024).

Urea and creatinine are used as biomarkers for renal function. The data shows that both values are elevated up to $50 \text{mg} \pm 1.50$, 1.5 ± 1.50 respectively in hyperlipidic induced rats, while reached it again to the normal ranges when treated with *Withania coagulans* herbal plant, same urea and creatinine levels are decreased in the results presented by (Sharma et al., 2017), while unlike with results presented by (Ojha et al., 2014)

Our findings regarding Superoxide Dismutase (SOD) align with previous studies (Afzal et al., 2024; Ojha et al., 2014) in which initially SOD level was decreased up to 1.90 ± 1.50 due to oxidative stress in hyperlipidic induced rats and again it reached significantly to the normal values when treated with herbal plant (*Withania coagulans*).

CONCLUSION

My research's findings are very comparable to those that have already been published. Triglycerides and LDL-C levels have significantly decreased, while HDL-C levels have significantly increased.

REFERENCES

1. Abbasi, S., Khan, A., & Choudhry, M. W. (2024). New insights into the treatment of hyperlipidemia: Pharmacological updates and emerging treatments. *Cureus*, 16(6). <https://doi.org/10.7759/cureus.63078>
2. Abdelhalim, M. A. K. (2010). The potential influence of high cholesterol diet-induced oxidative stress on composition and properties of red blood cells in rabbits. *African Journal of Microbiology Research*, 4(9), 836-843.
3. Ahmed, S., Bux Kachiwal, A., Khaliq, J., Shabir Barham, G., Ahmad, T., Bilal Khan, M., Kandhro, L., Ali Panhwar, I., Arif, M., Muhammad Gad, F., Faiz, H., Kandhro, B., & Ullah, E. (2024). Effect of prebiotic supplementation on the quality characteristics of rabbit meat. *Journal of Advanced Zoology*. <https://doi.org/10.53555/jaz.v45i6.5071>
4. Afzal, W., Naz, S., Ujan, J. A., Rind, K. H., Habib, S. S., Ullah, M., Zahid, M., Attaullah, S., Mohany, M., & Nazir, S. (2024). *Withania coagulans* root powder effect on growth, hematology, digestive enzyme activity, antioxidant status, serum immune response, and tolerance against *Aeromonas hydrophila* in Common Carp. *North American Journal of Aquaculture*, 86(4), 504-518. <https://doi.org/10.1002/naaq.10358>
5. Alloubani, A., Nimer, R., & Samara, R. (2021). Relationship between hyperlipidemia, cardiovascular disease and Stroke: A systematic review. *Current Cardiology Reviews*, 17(6), 52-66. <https://doi.org/10.2174/1573403x1699920121020034>
6. Arif, M., Kachiwal, A. B., Rehman, Z. ur, Soomro, S. A., khaliq, jahanzaib, Bilal, H., Ahmed, N., Dad, R. M., Abideen, Z. U., Zafar, H., Ahmed, S., Namood, S., Khan, M. B., Ali, M. M., & Ahmad, T. (2024). Studies On Influence Of Varying Age And Sex On Hematological Indices And Physiological Parameters Of Pateri Goat Breed Of Sindh. *Journal of Survey in Fisheries Sciences*, 11(1), 108-121. <https://doi.org/10.53555/sfs.v11i01.3020>
7. Bancroft, J. D., & Gamble, M. (Eds.). (2008). *Theory and practice of histological techniques*. Elsevier health sciences.
8. Bandawane, D. D., & Juvekar, A. R. (2022). STUDY OF ANTIHYPERGLYCEMIC, ANTIHYPERLIPIDEMIC AND ANTIOXIDANT ACTIVITIES OF WITHANIA

- COAGULANS FRUITS IN STREPTOZOTOCIN INDUCED NON-INSULIN DEPENDENT DIABETES MELLITUS IN RATS. *Indian Drugs*, 58(12), 63–71.
<https://doi.org/10.53879/id.58.12.12949>
9. Barquera, S., Pedroza-Tobías, A., Medina, C., Hernández-Barrera, L., Bibbins-Domingo, K., Lozano, R., & Moran, A. E. (2015). Global overview of the epidemiology of atherosclerotic cardiovascular disease. *Archives of medical research*, 46(5), 328-338.
<https://doi.org/10.1016/j.arcmed.2015.06.006>
 10. Chandra, H., Kumari, P., Bontempi, E., & Yadav, S. (2020). Medicinal plants: Treasure trove for green synthesis of metallic nanoparticles and their biomedical applications. *Biocatalysis and Agricultural Biotechnology*, 24, 101518.
<https://doi.org/10.1016/j.bcab.2020.101518>
 11. Deng, Y., Zhang, X., Shen, H., He, Q., Wu, Z., Liao, W., & Yuan, M. (2020). Application of the nano-drug delivery system in treatment of cardiovascular diseases. *Frontiers in bioengineering and biotechnology*, 7, 489.
<https://doi.org/10.3389/fbioe.2019.00489>
 12. Franco, M., Cooper, R. S., Bilal, U., & Fuster, V. (2011). Challenges and opportunities for cardiovascular disease prevention. *The American journal of medicine*, 124(2), 95-102.
<https://doi.org/10.1016/j.amjmed.2010.08.015>
 13. Gupta, R., Sonawane, T., & Pai, S. (2022). An overview on pharmaceutical properties and biotechnological advancement of *Withania coagulans*. *Advances in Traditional Medicine*, 22(4), 673-683.
<https://doi.org/10.1007/s13596-021-00558-7>
 14. Hennekens, C. (2000). Clinical and research challenges in risk factors for cardiovascular diseases. *European Heart Journal*, 21(23), 1917–1921.
<https://doi.org/10.1053/euhj.1999.2221>
 15. Jacobsen, A. P., Whelton, S. P., Blumenthal, R. S., & Mcevoy, J. W. (2024). Dyslipidemia. In *Hypertension* (pp. 476-488). Elsevier.
<https://doi.org/10.1016/B978-0-323-88369-6.00042-6>
 16. Khan, F. A., Kachiwal, A. B., Khaliq, jahanzaib, Soomro, S. A., Habibullah, Ahmad, T., Khan, M. B., Farooq, M. U., Namood, S., Faiz, H., Fayyaz, A., Raheel, M., Akhlaq, S., Khaliq, S., & Memon, M. (2024). Effect Of Supplementation Of B-Galacto-Oligosaccharide And Probiotic Mixture On Growth Performance Of Physiologically Stressed Rabbits. *Journal of Advanced Zoology*, 45(6).
<https://doi.org/10.53555/jaz.v45i6.5023>
 17. Kirichenko, T. V., Sukhorukov, V. N., Markin, A. M., Nikiforov, N. G., Liu, P. Y., Sobenin, I. A., ... & Aliev, G. (2020). Medicinal plants as a potential and successful treatment option in the context of atherosclerosis. *Frontiers in pharmacology*, 11, 403.
<https://doi.org/10.3389/fphar.2020.00403>
 18. Kopin, L., & Lowenstein, C. J. (2017). Dyslipidemia. *Annals of internal medicine*, 167(11), ITC81-ITC96.
<https://doi.org/10.7326/AITC201712050>
 19. Laaboudi, W., Ashmawy, N. S., El Hachlafi, N., Alshabrimi, F. M., Alnasser, S. M., Assaggaf, H., ... & Mrabti, H. N. (2025). Impact of olive tree extract on hyperlipidemia in rats: HPLC analysis of bioactive constituents. *CyTA-Journal of Food*, 23(1), 2463491.
<https://doi.org/10.1080/19476337.2025.2463491>
 20. Mainieri, F., La Bella, S., & Chiarelli, F. (2023). Hyperlipidemia and cardiovascular risk in children and adolescents. *Biomedicines*, 11(3), 809.
<https://doi.org/10.3390/biomedicines11030809>
 21. Ojha, S., Alkaabi, J., Amir, N., Sheikh, A., Agil, A., Fahim, M. A., & Adem, A. (2014). *Withania coagulans* fruit extract reduces oxidative stress and inflammation in kidneys of streptozotocin-induced diabetic rats. *Oxidative medicine and cellular longevity*, 2014(1), 201436.
<https://doi.org/10.1155/2014/201436>
 22. Otunola, G. A., Oloyede, O. B., Oladiji, A. T., & Afolayan, A. A. (2010). Effects of diet-induced hypercholesterolemia on the lipid profile and some enzyme activities in female Wistar rats. *African Journal of Biochemistry Research*, 4(6), 149–154.
<https://doi.org/10.5897/ajbr.9000099>
 23. Pala, R., Anju, V., Dyavaiah, M., Busi, S., & Nauli, S. M. (2020). Nanoparticle-Mediated Drug Delivery for the Treatment of Cardiovascular Diseases. *International Journal of Nanomedicine, Volume 15*, 3741–3769.
<https://doi.org/10.2147/ij.n.s250872>
 24. Rasool, A., Bhat, K. M., Sheikh, A. A., Jan, A., & Hassan, S. (2020). Medicinal plants: Role, distribution and future. *Journal of Pharmacognosy and Phytochemistry*, 9(2), 2111–2114.
 25. Shaito, A., Aramouni, K., Assaf, R., Parenti, A., Orekhov, A., El Yazbi, A., ... & Eid, A. H. (2022). Oxidative stress-induced endothelial dysfunction in cardiovascular diseases.
<http://dx.doi.org/10.31083/j.fbl2703105>
 26. Shazinosh, M. U. F., Namood, S., Marri, N. U., Khan, Z., Bismillah, A. U., & Kabir, A. (2024). Studies On Concentration of Some Milk Metabolic Enzymes at Different Parities, Stage of Lactation and Their Correlation with Composition and Yield of Milk in Red Sindhi Cows. *Journal of Survey in Fisheries Sciences*, 11(4), 175-188.
<https://doi.org/10.53555/sfs.v11i4.2902>
 27. Hemalatha, S., Sharma, S., & Joshi, A. (2017). Protective effect of *Withania coagulans* fruit extract on cisplatin-induced nephrotoxicity in rats. *Pharmacognosy Research*, 9(4), 354.
<https://doi.org/10.4103/pr.pr.1.17>
 28. Shukla, K., Dikshit, P., Shukla, R., Sharma, S., & Gambhir, J. K. (2014). Hypolipidemic and antioxidant activity of aqueous extract of fruit of *Withania coagulans* (Stocks) Dunal in cholesterol-fed hyperlipidemic rabbit model.
 29. Soetan, K. O., & Aiyelaagbe, O. O. (2009). The need for bioactivity-safety evaluation and conservation of medicinal plants-A review. *Journal of medicinal plants research*, 3(5), 324-328.
<https://doi.org/10.5897/jmpr.9001223>
 30. Su, X., Chen, X., & Wang, B. (2021). Pathology of metabolically-related dyslipidemia. *Clinica Chimica Acta*, 521, 107-115
<https://doi.org/10.1016/j.cca.2021.06.029>

31. Tripathi, D., & Pandey-Rai, S. (2019). Withania coagulans: An overview on therapeutic potential and use of recent biotechnological approaches for conservation and enhancement of secondary metabolites.
32. Vekic, J., Stefanovic, A., & Zeljkovic, A. (2023). Obesity and dyslipidemia: a review of current evidence. *Current Obesity Reports*, 12(3), 207-222. <https://doi.org/10.1007/s13679-023-00518-z>
33. Yang, R., Chen, D., Chen, Y., Ma, Y., Chen, C., & Zhao, S. (2025). Walnut oil prevents hyperlipidemia induced by high-fat diet and regulates intestinal flora and liver metabolism. *Frontiers in Pharmacology*, 15, 1431649. <https://doi.org/10.3389/fphar.2024.1431649>
34. Yoshikiyo, K., Shimizu, H., Nagato, E. G., Ishizuka, S., & Yamamoto, T. (2025). Comparative Analysis of γ -Cyclodextrin, Perilla Oil, and Their Inclusion Complexes on Liver Injury and Dyslipidemia Associated with Elevated Gastrointestinal 12-Hydroxylated Bile Acid Levels. *Molecules*, 30(2), 281. <https://doi.org/10.3390/molecules30020281>
35. Zhao, D. (2021). Epidemiological features of cardiovascular disease in Asia. *JACC: Asia*, 1(1), 1-13. <https://doi.org/10.1016/j.jacasi.2021.04.007>