Evaluation ofEthyl Acetate Extract of*Artocarpus lakoocha*Bark in the Management of Non-Alcoholic Fatty Liver Disease

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CERTIFICATE

This is to certify that Saptapadi Saha has carried out the research on the project entitled **"Evaluation of Ethyl Acetate Extract of *Artocarpus lakoocha* Bark in the Management of Non-Alcoholic Fatty Liver Disease"** under my supervision in the division of Pharmacology and Toxicology—Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032.

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DECLARATION

I declare that **"Evaluation ofEthyl Acetate Extract of*Artocarpus lakoocha*Bark in the Management of Non-Alcoholic Fatty Liver Disease"** is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

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PREFACE

The present study entitled "**Evaluation ofEthyl Acetate Extract of*Artocarpus lakoocha*Bark in the Management of Non-Alcoholic Fatty Liver Disease**" covers original research work conducted by the author for the award of Master of Pharmacy in the Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

Non-Alcoholic Fatty Liver Disease (NAFLD) is a global health concern affecting 25% of the population. This metabolic disorder, characterized by excessive liver fat accumulation, can lead to severe conditions like non-alcoholic steato hepatitis (NASH), cirrhosis, and hepatocellular carcinoma. Current therapeutic approaches mainly focus on lifestyle modifications and metabolic disorders, but these often yield limited success.Natural chemicals obtained from traditional medicinal plants have piqued attention as possible treatment agents for nonalcoholic fatty liver disease (NAFLD) in recent years. Artocarpus lakoocha, a tree species native to South and Southeast Asia, has a long history of use in traditional medicine systems for various ailments, including hepatic disorders. However, the scientific evidence supporting its efficacy in liver diseases, especially NAFLD, remains limited. This study aims to bridge this knowledge gap by conducting a comprehensive evaluation of the ethyl acetate Artocarpus lakoochaextract (ALE) in the context of NAFLD management. The multidisciplinary approach includes phytochemical analysis, in vitro and in vivo studies, and a preliminary clinical assessment to elucidate the extract's potential therapeutic effects and mechanisms of action. The findings may pave the way for developing new therapeutic strategies in NAFLD management, potentially offering a more holistic and sustainable approach to addressing this prevalent liver condition.

Keywords: Non-Alcoholic Fatty Liver Disease, Metabolic disorder, Non-alcoholic steato hepatitis, Artocarpus lakoocha, Ethyl acetate, Hepatic, Phytochemical analysis.

Saptapadi Saha

CONTENTS

DEDICATED TO MY FAMILY & MY MENTOR

INTRODUCTION

1.1 Epidemiology:

Excessive buildup of fat in the liver of people who drink little to no alcohol is a chronic liver illness known as nonalcoholic fatty liver disease (NAFLD). In advanced countries, it has grown into the most prevalent liver disease. In the same period, the prevalence rate jumped from 8.2% to 10.9%, and the overall count of cases with NAFLD grew significantly from 391.2 million in 1990 to 882.1 million in 2017 (Ge et al., 2020).The frequency of NAFLD, which encompasses fibrosis, cirrhosis, hepatocellular carcinoma (HCC), and nonalcoholic steatohepatitis (NASH), is rising as a result of poor physical activity and unhealthy eating behaviours(Sayiner et al., 2016). Because there are few approved treatments, erroneous disease progression prediction indicators, and inadequate screening of high-risk asymptomatic people, NAFLD is a common condition. While lifestyle changes are the primary form of treatment, other forms of therapy are also being considered. It is possible to lessen the impact of illness by better understanding the processes behind disease development, diagnostic biomarkers, and therapeutic intervention. The use of combination medicines is growing. The pathological characteristics, immune processes, and current treatment approaches for NAFLD will be defined in this study (Petagine et al., 2023).It indicates that males with type 2 diabetes and Obesity, with a mean age of 51.7 years, represent the phenotype of NAFLD patients worldwide. There is evidence of a stated linear rise in the incidence of NAFLD, diabetes, and metabolic diseases; this is particularly true in individuals with central Obesity, metabolic syndrome, and diabetes. Metabolic syndrome (40%) and hypertension (37%) are examples of metabolic comorbidities(Le et al., 2022). Up to 70% of people with type-2 diabetes mellitus (T2DM) also have NAFLD, and those with T2DM have a twice higher risk of dying from all causes. Patients with severe Obesity also have a 90% increased prevalence of NAFLD(Alexander et al., 2018).As a result, the prevalence of NAFLD represents a substantial worldwide health burden that has to be addressed by clinicians.

1.2Fatty liver disease:

Similar to the obesity pandemic, fatty liver disease is a common cause of elevated liver tests. The primary technique for determining the diagnosis or exclusion of fatty liver disease is liver biopsy assessment. Up to 5% of the mass of an adult liver may be made up of fat, with the size of the triglyceride (TGs) droplets suggesting the source of the buildup. Macrovesicular droplets usually do not cover the whole hepatocyte in cases of fatty liver disease, which is often characterized by large droplet steatosis or mixed large and tiny droplet steatosis(Brunt, 2007).Due to their increased caloric density and water-insoluble nature, TGs are the nutrient of choice for storage. Under normal conditions, they accumulate in different cell types, but in higher organisms, they are stored in adipocytes. For instance, vast amounts of TGs are stored in the liver of migrating birds for their lengthy seasonal travels; this liver has been utilized to make the delectable dish fois gras. Unhealthy liver diseases and serious clinical outcomes may result from people storing too many calories in their livers. The buffering of energy supply and demand variations is a critical function of TGs(Cohen, 2011).
Two significant causes of chronic liver disease globally are NAFLD and alcoholic liver disease (ALD). Both may result in cirrhosis and are distinguished by histological lesions, some of which include steatosis(Yeh & Brunt, 2014). 50% of deaths associated with cirrhosis are caused by alcohol, making it a primaryaetiology of ALD's last-stage liver disease.

20% of individuals with liver disease now use alcohol, making alcohol the most prevalent cause of cirrhosis in India (34.3%). Steatohepatitis, cirrhosis with consequences, and fatty liver are all included in ALD. Based on its reversible nature with abstinence and asymptomatic character, steatosis—which affects 90% of heavy drinkers—is deemed harmless.

Nevertheless, in comparison to abstinent controls, it signifies metabolic stress that lowers long-term survival and raises the chance of severe liver disease(Mitra et al., 2020). Adults are more likely to be diagnosed with NAFLD, which is defined by triglyceride buildup that exceeds 5% of liver weight. From simple steatosis to nonalcoholic steatohepatitis (NASH), which may advance to cirrhosis and hepatocellular cancer, it encompasses a variety of illnesses. Being caught early is critical since diagnosis is only achievable in the absence of substantial alcohol use.

De Ritis identified the ratio of aspartate transaminase (AST) to alanine transaminase (ALT) in serum, which is a sign of long-term problems in chronic viral diseases such as nonalcoholic fatty liver disease, alcoholism, and hepatitis approximately half a century ago. Long-term consequences, such as fibrosis and cirrhosis, are predicted by an increased AST/ALT ratio.

1.3 NAFLD :

In particular, the buildup of lipids in hepatocytes might change liver function due to genetic factors and other stressors. If these conditions continue, NASH may result from steatosis that induces inflammation. Advanced fibrosis, liver cirrhosis, and liver failure may ultimately arise from NASH. A sedentary lifestyle, Western food, Obesity, diabetes, impaired response of the body to insulin,and dysfunctionare the primary reasons behind NAFLD, a widespread hepatic disease in advanced countries. Fibrosis, cirrhosis, NASH, and hepatocellular cancer are possible outcomes. The aetiology of NAFLD is partly due to lipotoxicity in the liver, which causes inflammation of the fat tissueand internal dysbiosis. In NAFLD and other obesity-related illnesses, higher caloric intake is increasingly fueling metabolic inflammation(Kořínková et al., 2020). Patients with NASH often show no symptoms; high serum transaminases are the first indication of the condition during regular testing. Some people, nevertheless, could feel nausea, fatigue, and lose their appetite. More severe symptoms such as oedema, jaundice, and portal hypertension may occur in individuals if the illness advances to cirrhosis.

The first stage of NAFLD, sometimes called simple steatosis, is characterized by fat buildup in liver cells. NAFLD is another name for this condition. Finding it with imaging examinations or blood testing is possible, and it is often asymptomatic. The main treatments for simple steatosis are lifestyle changes like losing weight and being more active. Simple steatosis is usually harmless and avoidable. Metabolic syndrome, dyslipidemia, Obesity, and type 2 diabetes are risk factors. The risk of cardiovascular disease may rise with simple steatosis, even though it often does not result in liver damage(Friedman et al., 2018). (The liver fat buildup, inflammation, and hepatocyte destruction that define Nonalcoholic Steatohepatitis (NASH), a stage of NAFLD, are present. Stenosis, cirrhosis, and even hepatocellular cancer may develop as a result of it. Although the exact nature of the progression process is unknown, elements such as lipotoxicity, oxidative stress, and dysbiosis of the gut microbiota are probably involved. Usually, a liver biopsy is needed for the diagnosis, and weight reduction is the mainstay of lifestyle changes for care. The number of

pharmacological treatments is restricted; however, a number of innovative remedies are now being studied (Younossi et al., 2019).

A crucial stage of NAFLD called fibrosis is marked by an excessive buildup of extracellular matrix proteins—mainly collagen—caused by chronic liver damage. Liver tissue is gradually scarred as a consequence of this process, which is fueled by activated hepatic stellate cells. One crucial factor in predicting long-term results is the degree of fibrosis; advanced fibrosis raises the risk of liver-related morbidity and death. Although non-invasive techniques like elastography and serum biomarkers have enhanced the monitoring process,The most trustworthy method for accurate diagnosis remains liver biopsy. The most effective strategy for fibrosis regression is weight reduction, and management focuses on treating metabolic risk factors(Friedman et al., 2018).

The most severe stage of NAFLD, known as cirrhosis, is marked by a large amount of fibrosis and a deformation of the typical architecture of the liver. At this stage, there is a greater chance of hepatic decompensation, portal hypertension, hepatocellular cancer, and reduced liver function. Clinical characteristics, imaging tests, and non-invasive indicators are often used in the diagnosis process. A liver biopsy may be required for conclusive staging. The goals of management are to treat problems, stop more damage, and address metabolic risk factors. Liver transplantation may be considered in more advanced instances(Mahady & George, 2018).

- **Pathogenesis**

- **Lipid Accumulation**

The liver plays a crucial role in the metabolism, production, storage, and export of fats, as well as the import of free fatty acids (FFAs). Difficulties in any of these functions can result in the development of NAFLD(Berlanga et al., 2014). The liver absorbs free fatty acids primarily through the portal vein from dietary fatty acids and lipolysis in peripheral adipose tissues, particularly visceral and subcutaneous. The intricate control of free fatty acid storage and release within adipose tissues involves a sophisticated regulation by a variety of cytokines and hormones. These factors elicit distinct responses from adipose tissues, indicating a nuanced sensitivity to the complex interplay of regulatory signals. Liver FFAs are closely linked to visceral adipose tissues because they exhibit more sensitive lipolytic activities during fasting and exercise than subcutaneous adipose tissues. Thus, central Obesity- which is typified by a large amount of visceral adipose tissue, contributes significantly to the pathophysiology of NAFLD (Ono et al., 2010).

There is a buildup of lipids inside the hepatocytes of NAFLD, primarily in the form of Triglycerides. Free fatty acids and glycerol are esterified to produce triglycerides(Buzzetti et al., 2016). In the hepatocytes, TGs can be stored as lipid droplets or released into the bloodstream as very low-density lipoprotein. Apolipoprotein B is necessary to convert TGs into very low-density lipoproteins through esterification(Berlanga et al., 2014).Hepatic fat accumulation arises when there's an imbalance, with increased fatty acid uptake and de novo synthesis surpassing the rates of oxidation (Musso et al., 2009). In individuals grappling with type 2 diabetes mellitus or Obesity, the onset of insulin resistance triggers uncontrolled lipolysis within the adipose tissue.Non-esterified fatty acids (NEFAs) are ultimately

generated in large quantities and enter the hepatocytes as a consequence of this process, where the hepatocytes absorb them with the help of fatty acid transport protein FATP5, FATP2, and other protein transporter like caveolin-1, FA-building protein. CD36, also known as FA translocate, promotes NEFA uptake and intracellular trafficking in a variety of cells, such as macrophages, adipocytes, myocytes, hepatocytes, and enterocytes. The transcription factor forkhead box protein O1, insulin, and muscle contraction all promote FFA uptake by inducing CD3 translocation. Liver X receptor (LXR), Pregnane X receptor (PXR), and Peroxisome-activated receptor gamma (PPAR-γ) all share a common target in CD36, which has been demonstrated to increase in a murine model of hepatic steatosis. The precise involvement of CD36 in human diseases remains unclear; there is a notable association in morbidly obese individuals with NAFLD. It has been shown here that the quantity of CD36 mRNA is related to both the amount of liver fat and apoptosis(Arab et al., 2018).

In patients with NAFLD, the origins of hepatic triglycerides exhibit significant distinctions when compared to the general population. Specifically, only 60% of hepatic TG is from free fatty acid (FFA), with 25% attributed to hepatic DNL and an additional 15% derived from dietary fats (Méndez-Sánchez et al., 2007). DNL is a metabolic process primarily occurring in the liver and adipose tissue. This intricate series of metabolic events transpires within the cytoplasm, specifically the mitochondria, where surplus carbohydrates undergo conversion into fatty acids that are subsequently esterified into triglycerides. The initiation of DNL involves the transformation of acetyl-CoA into malonyl CoA facilitated by acetyl-CoA carboxylase, involving multiple cycles and enzymatic reactions. This malonyl CoA serves as a crucial substrate in the synthesis of 16-carbon palmitic acyl CoA, with the help of fatty acid synthase enzyme. Fatty acid synthase assumes a pivotal role in the intricate process of synthesizing fatty acid. Based on the state of metabolism, FAs are converted to TGs and either quickly metabolized or stored (Bullón-Vela et al., 2018). Transcriptional factors like sterol regulatory element binding protein-1(SREBP-1), peroxisome proliferator-activated receptor (PPAR-γ), and carbohydrate response element binding protein (ChREBP) can all be activated to increase hepatic DNL—different types of SREBP isoform exit: SREBP1c and SREBP2. SREBP2 regulates the cholesterol metabolism. When cellular cholesterol levels are reduced, SREBP2 becomes activated. It undergoes translocation from ER to the nucleus, and it exerts its influence on the transcription of specific genes associated with cholesterol synthesis, secretion, uptake, and transport. This regulatory process aims to enhance the availability of intracellular cholesterol (Musso et al., 2013). Insulin stimulates SREBP1c through LXR, which then increases fatty acid synthase transcription. ChREBP stimulates a liver type of pyruvate kinase transcription, which raises the synthesis of citrase, a substrate for the synthesis of fatty acid(Shimada et al., 2014).

Dietary factors significantly influence the development of NAFLD. The elevated intake of fat, characteristic of Western diets, has been linked to dyslipidemia, insulin resistance, and the onset of metabolic and cardiovascular disease (Fan & Cao, 2013). Fructose, unlike glucose, possesses the ability to influence hepatic lipid metabolism through direct activation of ChREBP and SREBP1c, along with a reduction in mitochondrial beta-oxidation. This dual mechanism ultimately promotes NAFLD formation(Pierantonelli & Svegliati-Baroni, 2019).

- **Insulin Resistance**:

In individuals with good health, when insulin binds to its receptor, The result is a series of events, resulting in the phosphorylation of various substrates, such as insulin receptor substrates (IRS)-1,2,3, and 4. These phosphorylated substrates play a crucial role in transmitting and amplifying the insulin signal within the body(Dowman et al., 2010). IRS proteins experience serine phosphorylation, a process that dampens insulin signalling by reducing the level of insulin-induced tyrosine phosphorylation (Malaguarnera et al., 2009). Various enzymes, including c-Jun-N-terminal kinase (JNK-1), kappa kinase beta (IKK-b), and protein kinase (PKC), play a role in promotingSerine phosphorylation of the insulin receptor substrate, resulting in a reduction in glucose uptake, decreased activation of glycogen synthase, and inhibition of the phosphorylation of forkhead box protein O (FOXO) are mechanisms that collectively lead to the stimulation of hepatic gluconeogenesis(Malaguarnera et al., 2009)(Polyzos et al., 2009).The inhibition of IRS1/2 signalling can be attributed to primary factors such as FFA, reactive oxygen species [ROS], endoplasmic stress [ER stress], and adipocyte-mediated alterations. The influences of IKK-b, JNK-1, and PKC play a pivotal role in this context. These elements collectively contribute to the hindrance of IRS1/2 signalling pathways(Marušić et al., 2021). Research findings have indicated that inflammatory cytokines, TNF-∝ hinders the effect of insulin-induced tyrosine phosphorylation of IRS1 by augmentation in the phosphorylation of Ser307(Rui et al., 2001). These outcomes are facilitated through the activation of the IKKβ-NF-κB pathway(Yuan et al., 2001). Elevated levels of TNF-∝ within adipose tissue could potentially contribute to the advancement of NAFLD by fostering systemic insulin resistance and triggering inflammation in diverse tissues(Fontana et al., 2007). The heightened secretion of IL-6 in adipose tissue, driven by JNK1 activity, led to an upregulation of hepatic SOCS3 expression. Consequently, this upregulated SOCS3 prompted the degradation of critical molecules associated with insulin signalling in the liver, ultimately culminating in the development of hepatic insulin resistance(Sabio et al., 2008). It has been shown that people with NAFLD have significantly higher blood levels of IL-6 (Coulon et al., 2012). Adipocytes also release distinct adipokines, including specialized leptin and adiponectin (Sun & Karin, 2012). Leptin significantly influences the dephosphorylation of IRS1(Cohen, 2011). Leptin levels in the blood are noticeably higher in NAFLD patients(Sakurai et al., 2021). Conversely, adiponectin plays a crucial role in improving NAFLD. This effect is mediated through AdipoR1 and AdipoR2. The hepatic AdipoR2 and AdipoR1 pathways have implications for triggering PPAR alpha and AMP-activated kinase, respectively, which boost insulin sensitivity(Yamauchi et al., 2007)—Hepatic DAG, a lipid mediator involved in TG production, is found in higher concentrations in livers associated with obesity. Under normal physiological circumstances, elevated TG levels are accompanied by a rise in DAG, which inhibits the action of the insulin receptor kinase(Pafili & Roden, 2021).

- **Indirect relation between diabetics and NAFLD**

NAFLD has a complicated and contradictory connection with antidiabetic drugs. An increased risk of acquiring or aggravating NAFLD has been linked to some antidiabetic medications despite the fact that diabetes is still a substantial risk factor for NAFLD. There is a reciprocal link between diabetes and NAFLD, with one illness raising the chance of the other. Diabetes is a risk factor for the advancement of NASH and cirrhosis, two more severe

types of NAFLD. Up to 70% of people with type 2 diabetes mellitus (T2DM) have NAFLD(Targher et al., 2021).Insulin, sulfonylureas, and thiazolidinediones (TZDs) are among the antidiabetic drugs that have been linked to the onset or progression of NAFLD. Increased lipogenesis, weight gain, modifications to lipid and glucose metabolism, and altered insulin sensitivity are all potential causes of NAFLD. That being said, not all antidiabetic drugs are harmfulto the liver. Metformin, SGLT2 inhibitors, and GLP-1 receptor agonists are among the more recent medication types that have shown possible advantages(Talamantes et al., 2023).The idea that antidiabetic drugs may primarily cause NAFLD emphasizes how crucial customized treatment is to the care of diabetic patients. Healthcare professionals need to carefully weigh the benefits and dangers of glycemic management for the liver. Regularly monitoring liver function and fat content in diabetic patients may be part of this. Patients at high risk for NAFLD may also benefit from using newer antidiabetic medications that may improve liver health. Lifestyle interventions, such as diet and exercise, can also improve liver health and diabetes control. Medication selection should be customized based on the unique characteristics of each patient, including body weight, liver function, and NAFLD risk factors(Kim & Lee, 2020).The physiological resemblances, genetic homogeneity, fast disease progression, well-characterized model, hormonal responses, histological similarities, features of the metabolic syndrome, responsiveness to interventions, availability, cost-effectiveness, ethical considerations, and versatility in study designs of Wistar rats make them an ideal model for studying metabolic disorders such as NAFLD. In addition to having NAFLD, they may also acquire other characteristics of metabolic syndrome and have well-characterized hormonal responses, as shown in scientific literature. Larger-scale research may benefit from the affordability and accessibility of Wistar rats. They do not, however, quite mimic every facet of human NAFLD, especially when it comes to the molecular pathways involved and the way the illness progresses over time(Ghezzi et al., 2012). The study that follows focuses on several variables that should be examined as a result of diabetes, including tissue antioxidants, serum variables, blood glucose level, blood cholesterol level, and histopathology of the affected organs.

- **NASH :**

NASH represents the evolving stage of NAFLD characterized by necroinflammation and varying levels of fibrosis. NASH is linked to subacute liver failure(Basaranoglu & Neuschwander-Tetri, 2006). The transition from NAFLD to NASH can be elucidated as a 'two-hit hypothesis'. In the initial phase, The building up of fatty acids in the liver is the earliest damage, intricately linked with insulin resistance. This accumulation not only heightens the liver cell's susceptibility but also paves the way for subsequent insults or injuries such as oxidative stress, mitochondrial dysfunction, lipid peroxidation, overproduction, and proinflammatory cytokines(Giorgio et al., 2013). The first hit hypothesis mentioned above is the pathophysiology of NAFLD.

In NASH, mitochondrial dysfunction is thought to be a combined effect of genetic and epigenetic factors(Caligiuri et al., 2016). The hub of free fatty acid β-oxidation predominantly takes place in the mitochondria of hepatocytes. Within the mitochondria, long-chain fatty acids undergo oxidation and subsequent transportation. This metabolic pathway is linked with the reduction of oxidized NAD+ and FAD to their respective reduced forms, namely NADH and FADH2(Fromenty et al., 2004). NADH and FADH2 undergo reoxidation

to NAD+ and FAD, transferring electrons to the mitochondrial respiratory chain. This process generates reactive oxygen species (ROS), contributing to oxidative stress if protective mechanisms are insufficient(Basaranoglu et al., 2013). Elevated FFA flux exhausts mitochondria, necessitating alternative sites for FFA processing, such as endoplasmic reticulum and peroxisomes. Mitochondrial exhaustion triggers oxidative stress via partially reduced oxygen molecules. Mitochondrial CYP2E1 attempts to oxidize surplus FFAs, intensifying ROS production. Excess FFAs are additionally oxidized in peroxisomes, where FADH2 and NADH electrons contribute to increased ROS formation. This process induces mitochondrial dysfunction, manifested by reduced proteins, DNA, and ATP (Noureddin & Sanyal, 2018). Impaired mitochondrial respiratory chain (MRC) function has the potential to initiate the generation of reactive oxygen species (ROS).When there is a disruption in the flow of electrons within the respiratory chain, the intermediates involved in the respiratory process may divert electrons towards molecular oxygen, resulting in the formation of superoxide anions and hydrogen peroxide (Wei et al., 2008).

An imbalance arising from elevated levels of ROS coupled with a reduction in antioxidants sets the stage for the lipid peroxidation of polyunsaturated fatty acids (PUFAs). The process of lipid peroxidation gives rise to cytotoxic aldehydes MDA and 4-HNE. The collaboration between ROS and aldehydescontributes to oxidative stress aggravating, concurrently promoting elevated levels of inflammatory cytokines. These events play a pivotal role in instigating hepatic inflammation, ultimately contributing to the emergence of various hepatic lesions associated with NASH(Basaranoglu & Neuschwander-Tetri, 2008). Reactive oxygen species (ROS) activation triggers the nuclear factor-κB, leading to the initiation of TNF-α synthesis. This activation also results in increased expression levels of many cytokines, including Fas ligand, TNF-α, IL-6, IL-8, and TGF-β(Rolo et al., 2012).

Additionally, the activation of death receptors is linked to Fas-ligand, which has been demonstrated to be correlated with heightened necrosis through the activation of caspases and the induction of cell death via Fas-ligand. An erratic cytokine profile and increased TNF-α receptor expression in the liver exacerbate TNF-α's effects on NASH. This leads to a rise in theprocess of mitochondrial membrane lipid peroxidation, which worsens their functioning and intensifies oxidative stress. TNF-α induces inflammation by activating NADPH oxidase, which triggers TNF receptor-1 expression (Ucar et al., 2013). NF-κB has been proposed as a pivotal contributor to the escalation of inflammation in the liver(dela Peña et al., 2005).

- **Treatment:**

Modifying one's lifestyle and controlling related metabolic disorders are the mainstays of therapy for NAFLD. Though a few strategies have shown potential, there aren't any FDA-approved drugs, particularly for NAFLD, at this time. An outline of current treatment approaches is provided below:

- **Lifestyle Modifications:**

- **Weight loss:** A 7-10% reduction in body weight can significantly improve liver histology in NASH patients.
- **Diet:** Mediterranean diet and low-carbohydrate diets have shown benefits.
- **Exercise:** Regular physical activity, both aerobic and resistance training, can reduce liver fat(Vilar-Gomez et al., 2015).

- **Management of Metabolic Comorbidities:**

  - Treating diabetes, hypertension, and dyslipidemia can improve NAFLD outcomes.
  - Metformin, while not directly improving liver histology, may reduce the risk of hepatocellular carcinoma (Mantovani et al., 2018).

  - **Pharmacological Interventions:**

  - Vitamin E: Shows some benefits in non-diabetic NASH patients.
  - Pioglitazone may affect both diabetes and non-diabetic individuals' liver histology should be improved.
  - GLP-1 receptor agonists: Emerging evidence supports their use in NASH(Chalasani et al., 2018).

- **Emerging Therapies:** Several drugs are in various stages of clinical trials, targeting different pathways:

  - FXR agonists (e.g., obeticholic acid)
  - PPAR agonists
  - CCR2/CCR5 antagonists
  - FGF21 analogues(Friedman et al., 2018)

- **Liver Transplantation:**For those with hepatocellular carcinoma or end-stage liver disorders brought on by NAFLD(Younossi et al., 2016).

- **Naturally obtained Phytochemicals:**

Phytochemicals obtained from plants are one of the most important natural sources of inspiration for medication development. These chemicals, produced for specific purposes, can act as a natural template for the development of novel medications. A minimum of 12,000 such compounds have been identified, accounting for less than 10% of the total. The varied chemical structures of these compounds could serve as foundational scaffolds for the development of future drugs(Lai & Roy, 2004).

Over the past 25 years, there has been a growing interest in harnessing phytochemicals as a basis for drug discovery(Newman & Cragg, 2007). Approximately 60% of all pharmaceuticals used in clinical settings worldwide are made up of natural materials or their derivatives, with medicinal plants accounting for 25% of all medications(Gurib-Fakim, 2011).

More than 28 percent of newly launched chemical entities in the market have their origins in natural items. According to research on plants, between 250,000 and 350,000 plant species have been discovered, of which roughly 35,000 are used medicinally worldwide (Kong et al., 2003). The World Health Organization (WHO) reports that 85% of plant-based traditional medicines are utilized by 65% of the global population and 80% of people in developing countries, indicating the importance of plant-based medical systems(Cragg & Newman, 2013).

People in rural regions can receive primary healthcare services via the usage of medicinal plants. According to a 2004 survey, between 40 and 50 percent of individuals in wealthy nations use traditional medicines, whereas 80% of the individuals in third-world countries depend on them for their fundamental healthcare requirements. These conventional medicines are primarily plant-based treatments (Oyebode et al., 2016).

LITERATURE REVIEW

(Pandey & Poonia, 2021) Native to South and Southeast Asia, *Artocarpus lakoocha*, sometimes called monkey fruit or lakoocha, is a species of tropical tree in the Moraceae family. It is well-known for its traditional applications and therapeutic qualities. This review of the literature offers an overview of recent studies on A. lakoocha, including its pharmacological properties, traditional applications, phytochemistry, and botanical features. The fruit, wood, and medicinal qualities of this tree are highly prized. The purpose of the study is to provide a thorough assessment of the phytochemical components, pharmacological activity, traditional applications, and botanical features of the plant.

(Bishnoi et al., 2017) The medium-to-large evergreen tree Artocarpus lakoocha has big, elliptic obovate leaves and rough, dark brown bark. It has tiny, unisexual blooms that are found on the same tree as both male and female flowers. It may reach a height of up to 25 meters. The fruit also referred to as "monkey jack," is a big, meaty syncarp with many seeds. Male and female blooms grow on different trees in this dioecious tree.

(Sitorus et al., 2022) Research on the phytochemical makeup of A. lakoocha has identified a number of bioactive substances, including phenolic substances, dihydromorin, artocarpesin, and oxyresveratrol. These substances are linked to flavonoids, tannins, saponins, and stilbenoids and exhibit several different biological activities. Potentanti-inflammatory and antioxidantoxyresveratrolhas been thoroughly investigated for its medical uses, particularly in the treatment of neurological illnesses and hyperpigmentation disorders.The bark extract of A.lakoochacontains steroids, tannins, and flavonoids. It has been discovered that the seeds contain fatty acids, the main ones being oleic, stearic, and palmitic acids.

(Hawari et al., 2021) Flavonoids are a group of secondary metabolites found in Artocarpus lakoocha that are well-known for having antibacterial, anti-inflammatory, and antioxidant qualities. Research has separated the flavonoids from the tree's leaves and bark, including quercetin, kaempferol, and their glycosides.

(Mahamud et al., 2023) Oxyresveratrol is a naturally occurring stilbene derivative found in Artocarpus lakoocha that has potent anti-inflammatory and antioxidant qualities. It also scavenges free radicals and guards against oxidative stress by preventing the production of proinflammatory mediators.

(Hossain et al., 2016) Asian societies have long used the Artocarpus lakoocha tree, which is prized for its nutritional and therapeutic qualities. The fruit is eaten raw or cooked, and its wood is used to make furniture and other building materials. Traditional medical practices such as Ayurveda and Thai medicine make use ofthe bark, fruits and leaves of the plant. It is thought that the plant has antibacterial, anthelmintic, anti-inflammatory, and antioxidant effects. While latex is used to cure skin diseases, bark decoction is utilized to treat gastrointestinal issues. The heartwood extract, called "puag-haad," is used as a taenicide and to cure diseases caused by tapeworms in Thailand.

(Saesue et al., 2024) The potent radical scavenging activity of oxyresveratrol and flavonoids found in Artocarpus lakoocha is credited with its antioxidant properties. This compound also reduces oxidative stress and shields cellular components from damage.

(Hankittichai et al., 2020) The anti-inflammatory plant Artocarpus lakoocha is thought to have the ability to cure inflammatory disorders because of its oxyresveratrol concentration, which prevents the generation of enzymes and cytokines that promote inflammation.

(Sitorus et al., 2022) With its flavonoids, tannins, and other phenolic components, the antimicrobial Artocarpus lakoocha tree has been shown in many preparations to suppress harmful microorganisms such as bacteria and fungi.

Plants rich in phytochemicals, especially oxyresveratrol, which has a variety of pharmacological properties, include Artocarpus lakoocha, which has essential nutritional, medical, and economic significance. Its traditional applications are supported by scientific study, which also suggests that novel medicinal agents might be developed. To thoroughly investigate its possibilities, further research is required.

AIM,OBJECTIVE& RATIONALE

Aim: To examine the Pharmacological evaluation of extract obtained from the bark of *Artocarpuslakoocha*.

Objective:

1. To evaluate the hepatoprotective effect of ALE extract against the NAFLD model.
2. To evaluate the therapeutic effect of ALE on the liver by measuring such biomarkers, e.g., Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Low-density lipoprotein (LDL), High-density lipoprotein (HDL), Triglyceride (TG), Total cholesterol (TC).
3. In vitro evaluation of hypoglycemic activity
4. To determine the antioxidant potential of ALE.
5. To observe the histopathological changes in experimental rats.

Rationale:

- Few drugs are available to manage NAFLD; most medicines that may help are being studied in clinical trials.
- Natural products can be the ultimate solution with fewer side effects.
- Scientific literature suggests that *Artocarpus lakoocha*has antioxidant, anti-inflammatory action, and hypoglycemic properties.

PLAN OF WORK

I) Literature review and selection of plant

II) Collection and identification of Artocarpus lakoocha bark

III) Extraction of the selected plant

IV) Preliminary phytochemical and physicochemical study of the extract

V) Phytochemical characterization of the extract

a) DPPH

b) NO

c) TPC

d) TFC

VI) Evaluation of in vitro antidiabetic potential

a) α-amylase

b) α-glucosidase

VII) Induction of NAFLD in rats

VIII) Evaluation of in vivo hepatoprotective potentiality

IX) Evaluation of Serum Liver Function Test

a) ALT or SGPT level

b) AST or SGOT level

c) Blood glucose level

X) Evaluation of serum lipid

a) TC, TG, LDL, VLDL, HDL

XI) Histopathology

XII) Tissue antioxidant parameters

a) Lipid peroxidation (MDA)

b) Catalase

c) Reduced glutathione (GSH)

PLANT PROFILE

Plant Profile

5.1 Name: *Artocarpuslakoocha*

 **5.2 Plant Taxonomy:** ***Artocarpus lakoocha***

Kingdom: Plantae

Class: Equisetopside

Order: Rosales

Family: Moraceae

Genus: Artocarpus

Phylum: Tracheophyta

5.3 Synonyms:

Artocarpusficifolius

Artocarpus yunnanensis

Artocarpus cumingianavar, Artocarpus mollies Miq., Artocarpus ovatus Blanco

5.4 Local name:

Launch, Barhal, Dahu, Myauklok, Hat lom, Hat non.

Hindi: Barhal

Bengali: Deuwa or barrar

English: Monkey jack, Lakoocha

Kannada: Naka-renu

Tamil: Tinippalavi

Telugu: Lakuchamu

Assamese: Bohot

5.5 Parts used: Bark, Leaves, Fruit, Roots and Latex.

5.6 Ethnobotany:

Medicinal Uses: Anti-inflammatory, antiviral, anticancer, and anti-HIV characteristics are therapeutic qualities of A. lakoocha. Its seeds and milky latex are employed as purgatives, and its pulp functions as a refresher for the liver. The tannin in the bark of the tree is used to heal wounds and sores on the skin. β-amyrin acetate and lupeol acetate, which are present in the fruit, may have hypolipidemic and antihyperglycemic properties. While the hydroglycolic extract is utilized as an efficient and cost-effective skin-whitening treatment, the juice, seeds, bark, root, and leaves are employed as purgatives. In traditional Thai medicine, the herb is also utilized for anti-ageing and anti-inflammatory treatments(Hossain et al., 2016).

Culinary Uses: Curries, pickles, and delectable sauces are made from various parts of these fruits and the spike of the male flower. The plant's stem is used to make firewood and lumber, and its leaves are fed to animals.

5.7 Morphology:

Artocarpus lakoocha (A.lakoocha), a deciduous tree, extends upward to heights ranging from 15 to 18 meters, showcasing a wide-spreading canopy(Vanajakshi et al., 2016).

Leaves: The leaves are arranged alternately, ranging from 10 to 25 cm long. They take on an elliptical shape with pointed tips and possess a leathery texture(Gautam & Patel, 2014). The leaves exhibit a distichous arrangement, where the angle between the first and second and the third and fourth leaves is 120^{o}, while between the second and third, and the fourth and fifth leaves, it's 240^{o}, continuing in this pattern. Consequently, the dorsal leaves are spaced at half the interval compared to those on the ventral side of the branch(Sharma, 1962).

Bark: The colour of the bark is light brown to dark brown (Islam et al., 2018). The slash is deep red with milky latex(Orwa, 2009).

Flower: The flowers display a typical irregularly round shape, boasting a velvety yellow surface. Male blooms present a vibrant orange-yellow hue, while their female counterparts lean towards a captivating reddish tone. On a single tree, one can observe the presence of both male and female flowers(Gupta et al., 2020).Within the male flower, a lone stamen stands as its emblem, enveloped by the perianth, which undergoes rapid expansion and forms an elegant arch over the stamen. Contrarily, in the female catkin, the perianth's growth exhibits asymmetry as it envelops the pistil, with its style extending outward from the blossom during maturity(Banerji & Hakim, 1954).

Fruit: The syncarp structure of fruit entails the culmination of the entire female inflorescence into a single cohesive entity(Orwa, 2009). The mature fruit displays hues ranging from pinkish to brownish, often tinged with a hint of yellow,while showcasing an irregularly rounded shape spanning 5 to 12 centimetres in diameter.Its surface is adorned with a velvety texture, distinguished by sparse soft spikes in brown-black shades.Fruit exhibits a fibrous and elastic texture, typically containing between 10 and 30 seeds whose size and shape are influenced by the maturity of the fruit(Pandey & Poonia, 2021).

Seed: The seeds display irregular shapes and sizes akin to the fruits they originate from. Upon reaching maturity, they typically measure around one centimetre, possessing a flattened structure with a pointed tip at the end where the embryo resides(Orwa, 2009). Seeds harbouring a sticky white latex are notably recalcitrant, complemented by their thin, pale seed coats(Bishnoi et al., 2017).

5.8 Geographic Distribution:

Artocarpus lacoocha is a widespread distribution species. Artocarpus species are mainly found in Asia's tropical and subtropical zones. The Indian rainforests are thought to be the birthplace of Artocarpus heterophyllus, or jack fruit. Within the tropical areas of south and southeast Asia, namely in Nepal, Sri Lanka, India, Myanmar, Indonesia, Vietnam, and Thailand, Artocarpus lacoocha is a widespread distribution species (Vanajakshi et al., 2016).

5.9. Uses:

The plant is used for anti-inflammatory activity, antioxidant activity, Pancreatic lipase inhibitory activity, antidiarrheal activity, analgesic activity, antibacterial activity, cytotoxic activity, antihelmintic activity, insecticidal activity(Vanajakshi et al., 2016).

5.10. Chemical Constituents :

The heartwood contains artocarpin, nor-artocarpin, norcycloartocarpin, cycloartocarpin, resorcinol, and oxyresveratrol, β-sitosterol(Vanajakshi et al., 2016).Membrane application was used to extract the neuroprotective component oxyresveratrol from the extract solution of A. lakoocha. The fruit contains steroids, lignins, tannins, flavonoids, and phenols. The agglutinins (ALA I and ALA II) in lakoocha seeds have an intensehaemagglutination activity and are purgative(Bhattacharya et al., 2019).

COLLECTION, IDENTIFICATION, PHYTOCHEMICAL CHARACTERIZATION

6.1 Soxhlet extraction:

One hot continuous extraction method is soxhlet extraction, in which a dried crude drug is subjected to constant extraction with the same solvent. The soxhlet extractor primarily consists of three parts:

1. At the top condenser. Here, the solvent vapour gets condensed and drips into the Soxhlet extractor's main chamber.

2. In the middle is the Soxhlet apparatus's main chamber, which contains the Thimble. The main chamber is loaded with the Thimble, which is the powdered crude drug wrapped in a piece of filter paper.

3. At the bottom- a round-bottom flask. As it is more popularly known, it holds the solvent or menstruum. A sufficient amount of solvent necessary for extraction is loaded in this flask.

The solvent is boiled in the round-bottom flask with the help of a heating mantle. The temperature is set at a temperature around the solvent's boiling point. One side tube originating from the round-bottom flask opens at the top of the main chamber of the soxhlet apparatus and carries the solvent vapours to the condenser. Another thin tube, the siphon tube, is connected to the main chamber. As the solvent level in the chamber reaches the top of the siphon tube, the entire solvent is transferred into the round-bottom flask. The colour of the solvent in the side tube should be monitored continuously. As long as any tinge of colour is visible in the siphon tube, the extraction should be continued. When the solvent in the side tube gets colourless, the liquid containing the phytoconstituents should be collected and evaporated in a vacuum. If any solid residue is visible, the extraction should be continued. Otherwise, the extraction procedure need not be continued. The importance of this technique is that the process can be done multiple times to achieve effective extraction.

After the extraction, a finished extract is obtained through several downstream processing methods. First, the solvent is removed using a suitable method, which is referred to as concentration. After the concentration procedure, the plant extract is subjected to phytochemical analysis. The preliminary qualitative phytochemical analysis detects the presence of secondary metabolites. The quantitative estimations of the total amount of a particular kind of secondary metabolite, such as the TPC and TFC, are also under the scope of the present study.

6.2 Collection and identification

The whole plant was collected in November 2023 from Birati, Kolkata, West Bengal. The plant material was identified as *Artocarpus lakoocha*, a member of the Moraceae family, by taxonomist R. K. Gupta, Scientist-E & Head of the Central National Herbarium at the Botanical Survey of India (BSI). An authenticated voucher specimen (JU/TB-02) of *Artocarpus lakoocha* was deposited in the BSI herbarium on December 21, 2023.

1 **2** **3**

Figure (1-3): Parts of *Artocarpus lakoocha* (1 – Leaves, 2- Root and Stem, 3- Twig)

6.3 Processing of *Artocarpus lakoocha*

The bark part of*Artocarpus lakoocha*was cleaned with tap water and sanitized. The bark was then shade-dried until dryness at room temperature (25ºC±2). After proper drying, the plant materials were ground into a fine powder to conduct further experiments.

6.4 Extraction

In a Soxhlet apparatus, the AL bark was extracted using ethyl acetate. The extract was filtered, and the previous step was repeated twice. The solvent was fully evaporated under decreased pressure using a rotary evaporator, after which the concentrated extract was lyophilized and kept for later use in vacuum desiccators at 20°C. The yield value was about 12.6% w/w**.**

6.5 Standardization of *Artocarpus lakoocha (*AL**):**

6.5.1 Determination of physicochemical parameters:

I. **Moisture content determination**:

To eliminate moisture from the plant material, one gram of powder was put on a flat Petri dish and heated to 105°C ina hot air oven.The dried samples were weighed after cooling at room temperature inside a desiccator. The percentage of moisture content was determined for the previously weighed powdered drugs(Bhatti et al., 2015).

$$\text{Total moisture content (\%)} = \frac{\text{Weight of dried sample}}{\text{Weight of sample taken}} \times 100$$

II. **Total ash content:**

Two grams of dried powder plant material were weighed in a silica crucible. The powder material was After two to three hours at 450°C in a muffle furnace to remove all of the carbon, it was cooled in a desiccator. Next, the powder was weighed to make sure it was complete ashing. For thirty minutes, it was heated in the furnace once again. It was cooled and weighed.The weight of the ash was compared with the weighed powdered material (Bhatti et al., 2015).

$$\text{Percentage of ash} = \frac{\text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

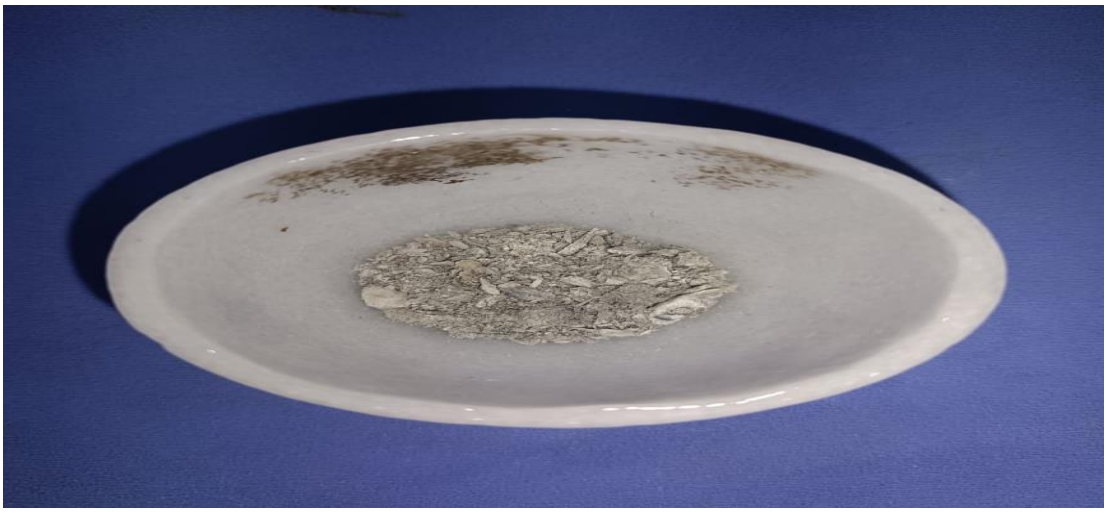


Figure 4: Ash Value

III. **Determination of water-soluble ash:**

Water was eliminated in order to digest the resulting ash. Twenty to thirty minutes were spent boiling 25 millilitres of solvent in a water bath. Whatman filter paper, devoid of ash, was used to filter the contents of the crucible. After being cautiously taken out of the crucible without causing any harm, the residue-filled filter paper was folded and put back inside. After that, it was burnt for an hour at 450°C in a muffle furnace and dried in a hot air oven. The water was chilled using a desiccator before the final weight was noted. The percentage of the water-soluble ash was calculated using the previously weighed powdered medicine(Bhatti et al., 2015).

$$\text{Percentage of soluble ash} = \frac{\text{Weight of soluble ash}}{\text{Total weight of ash}} \times 100$$

IV. Determination of acid insoluble ash value:

After boiling the previously weighed total ash for 15 minutes with 25 ml of 2N HCl, the mixture was filtered through ashless filter paper.After filtration, the filter paper with ash was placed in a silica crucible and ignited at 450ºC in a muffle furnace until the ash became carbon-free. The crucible was then allowed to cool to room temperature, and the total weight of the acid-insoluble ash was computed using the weight of the previously consumed powdered medication(Bhatti et al., 2015).

$$\text{Percentage of acid insoluble ash} = \frac{\text{Weight of acid-insoluble ash}}{\text{Total weight of ash}} \times 100$$

6.5.2 Determination of extractive value:

The dry powdered plant material of *A.lakoocha* was extracted with water, methanol, ethanol, acetone, chloroform and petroleum ether. 1 g of the coarsely powdered plant material was weighed in a weighing bottle and transferred into a dry conical flask. Then, the flask was filled with solvent (15 ml) separately. The flasks were corked and kept aside for 24 h at room temperature, shaking frequently. The mixtures were filtered into a measuring cylinder through Whatmann No. 1 filter paper. After the filtration, it was then transferred into a weighed Petry plate. The obtained extracts were concentrated to dryness by keeping the filtrate for complete solvent evaporation. The extractive value in percentage was calculated and recorded using the following formula—the ethyl acetate extract of AL(Wado et al., 2022).

$$\text{Extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

- **Preliminary phytochemical screenings of *Artocarpus lakoocha***

The preliminary phytochemical screening of the aqueous extract of *A. lakoocha* was carried out using various standard methods (Khandelwal, 2008), which are mentioned below.

6.6: Determination of phytochemical tests:

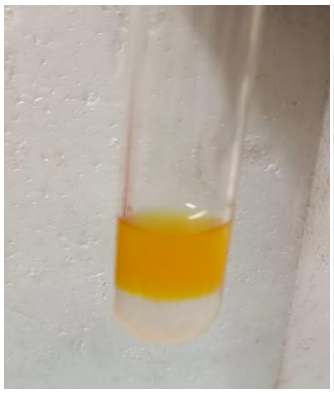
Because medicinal plants include phytochemical components, they may be used to treat and even cure diseases in humans(Nostro et al., 2000). Plant substances known as phytochemicals are non-nutritive but have protective or disease-preventive qualities. According to current research, the same molecules that plants make to protect themselves also serve to protect them against disease (Ajuru et al., 2017). Therefore, it is crucial to identify the

phytoconstituents in an ant substance or its extract. Alkaloids, flavonoids, saponins, tannins, steroids, glycosides, and carbohydrates were divided into chemical groups.

6.6.1 Test for Triterpenoid

- **Salkowski Test**(BOSILA & EL-SHARABASY, 2009)

1 ml of concentrated H_2SO_4 was added to 10 mg of extract diluted in 1 ml of chloroform. The presence of steroids was shown by the reddish-blue hue of the chloroform layer and the green fluorescence of the acid layer. But there is an absence.



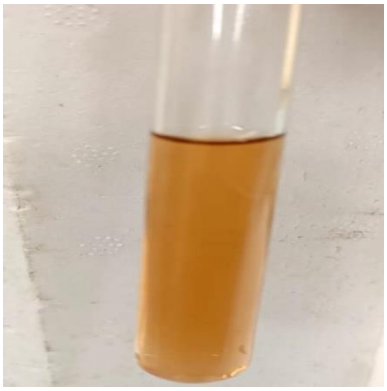
5. Salkowski Test

6. Shinoda Test

7. Xanthoprotein test

8. Benedict test

9. Quinones Test



10. Resin Test

Figure (5-10): Different phytochemical test result

6.6.2 Test for Flavonoids

- **Lead acetate solution test:**
  - Test the sample solution in water or ethanol, add 10% lead acetate, and observe for changes. A yellow precipitate indicates flavonoid presence(Harborne, 1998).

- **Shinoda test:**(Munde et al., n.d.)

  - In alcohol, a small amount of extract was dissolved. Two to three pieces of magnesium were added, then powerful hydrochloric acid was added, and the mixture

was boated. Appearance The appearance of magenta indicates the presence of flavonoids.

- **H_2SO_4 test:**

  - Prepare a sample extract using solvent, add 1-2 mL to a test tube, add concentrated sulfuric acid, and observe colour change at the interface(Yadav et al., 2023).

6.6.3 Test for Tannins(Aynilian et al., 1971)

- 1 mL of 5% ferric chloride solution was combined with 5 ml of extract solution. The greenish-black colour suggested the presence of tannins.
- To 5 ml. of extract, 1 mL of 10% aqueous potassium dichromate solution was added. The production of a yellowish-brown precipitate revealed the presence of tannins.
- To 5 ml. of extract, I mL of a 10% lead acetate solution in water was added. The emergence of yellow precipitate indicated the presence of tannins.

6.6.4 Test for Glycoside(Kanekar & Kanekar, 2022)

- **Kellar kiliani test:**

  - In a test tube, add a small sample, ferric chloride solution,glacial acetic acid, and concentrated sulfuric acid. Observe the interface for colour changes and observe the reaction(Rathore et al., 2012).

- **Bontrager's test:**

  - One millilitre of extract solution was treated with a few millilitres of mild sulphuric acid before being filtered and chloroform extracted. The layer was treated with 1 ml. of ammonia. The formation of a crimson colour indicates the presence of anthraquinone glycosides.

6.6.5 Test for Carbohydrates

- **Benedict's test**(Bhandary et al., 2012)

  - In order to search for the production of a reddish-brown precipitate, which would indicate the creation of carbohydrates, the test solution was heated in a water bath along with a few drops of Benedict's reagent, an alkali solution containing a cupric citrate complex.

- **Molish test:**(Dada & Adebayo, 2021)

  - 1 mL of a-naphthol solution and 1 mL of concentrated sulphuric acid were added to 2ml of extract via the test tube sides. Carbohydrates are detected by the presence of a purple or reddish-violet tint at the junction of the two liquids.

- **Fehling's test:**
  - Prepare the sample solution by dissolving the test substance in water or diluting it. Mix Fehling's solution A and B in a clean tube. Add the sample solution and heat in a water bath for 5-10 minutes (Nielsen & BeMiller, 2010).

6.6.6 Test for Alkaloids(de Oliveira Souza et al., 2020)

- **Mayer's test:**
  - A 1.2 ml. test tube held the extract. When 0,2 ml. of weak hydrochloric acid and 0.1 mL. of Mayer's reagent are mixed, a yellowish buff precipitate forms, indicating a positive test for alkaloids. Dragendroff'stest.
  - 0.1 ml of dilute hydrochloric acid and 0.1 ml of Dragendroff's reagent were added to a test tube containing a 2m1 extract solution. The production of orange-brown precipitate confirmed the presence of alkaloids.
- **Wagner's test:**

- Two millilitres of extract solution were treated with 0.1 millilitres of Wagner's reagent and two millilitres of mild hydrochloric acid. A reddish-brown colouration indicated a positive response to alkaloids.

- **Hager'stest:**

- The extract was combined with 0.2 millilitres of mild hydrochloric acid and 0.1 millilitres of Hager's reagent. A yellowish precipitate suggested the presence of alkaloids.

6.6.7 Test for Phenols

- **$FeCl_3$ test:**

- The test solution received 3-4 drops of $FeCl_3$. The creation of a bluish-black colour indicates the presence of phenol (Saxena & Kumar, 2020).

- **Lead acetate test:**

- In the test tube, extract mixed with a few drops of 10% lead acetate solution to the test tube. The formation of a white precipitate indicates the presence of phenolic compounds(Kumar, 2014).

6.6.8 Test for Saponin(Khandelwal, 2008)

- **Frothing test:**

- Test the foam formation by adding 1 gram of powdered sample or plant extract to 10 mL of distilled water, shaking vigorously for 30 seconds, and allowing it to stand undisturbed for 30 minutes.

6.6.9 Test for Protein test

- **Millon's Test:**

- Add Millon's reagent to 2-3 mL of protein solution, mix well, heat, and observe colour change (Kamineni et al., 2016).

- **Xantho Protein test:**

- In a test tube, mix 2-3 mL of protein solution with concentrated nitric acid (HNO_3), heat, and cool. Add excess ammonium hydroxide solution and observe any colour change (Jagtap & Jadhav, 2019).

6.6.10 Test forPhlabotanin test

- A test tube or beaker is used to observe the colour change of a prepared extract when 1% ferric chloride ($FeCl_3$) is added. A positive result is indicated by the formation of a blue, green, or blackish-blue complex due to the reaction between tannins and the ferric chloride solution(Ogbuanu et al., 2020).

6.6.11 Test for Anthraquinones

- A test tube contains extract, chloroform, and 10% sulfuric acid. Mix thoroughly and let stand for 10-15 minutes. A positive result is indicated by a colour change in the lower chloroform layer, typically pink, red, or orange, indicating the presence of anthraquinones(Harborne, 1998).

6.6.12 Test for Quinones

- A test tube contains extract with alcoholic KOH solution. Colouration ranges from red to blue (Ogbuanu et al., 2020).

6.6.13 Test for Resins

- To detect resin, add concentrated sulfuric acid to the residue in an evaporating dish and observe the reaction. A characteristic colour change, like deep blue or green, indicates the presence of resin. To confirm, dissolve a small portion of the residue in acetone and add dilute sulfuric acid to the solution. Observe any colour changes or precipitate formation(Dungani et al., 2014).

6.7Preparation and characterization of tincture

A total of 21 amber-colored bottles with 10 ml capacity were used. To these, 10 ml of alcohol with varied strengths—40, 50, 60, 70, 80, and 90 percent (V/V) and absolute alcohol were added. Each of the twenty-one bottles contained 1g of*Artocarpus lakoocha* bark powder. Each batch was allowed to macerate for a specific number of days: 7, 14, and 21. The materials were filtered through a sintered glass crucible (G4) while under reduced pressure after the required amount of maceration time. Physical characteristics were investigated, including colour, pH, specific gravity, and total solid content(Barman et al., n.d.).

Figure 11: **Tinctures of different alcohol concentrations after maceration for differing periods**

EXPERIMENT WORK OR METHODOLOGY

7.1 Drugs and Chemicals

Standard Ascorbic acid(Sisco Research Laboratories Pvt.Ltd), Gallic acid (Sisco Research Laboratories Pvt.Ltd), Quercetin (Sisco Research Laboratories Pvt.Ltd), dibasic sodium phosphate, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Sisco Research Laboratories Pvt.Ltd), Sodium phosphate dibasic dihydrate ($Na_2HPO_4.2H_2O$) (Avra Synthesis Pvt.Ltd), Potassium dihydrogen phosphate (KH_2PO_4) (Avra Synthesis Pvt.Ltd), Hydrogen Peroxide (H_2O_2) (35% in water)**(TCI)**, Folin cio-calteau phenol reagent (Sisco Research Laboratories Pvt.Ltd),Aluminium chloride ($AlCl_3$) (Avra Synthesis Pvt.Ltd),Gallic acid (Sisco Research Laboratories Pvt.Ltd), Sodium Nitrite ($NaNO_2$) (Sisco Research Laboratories Pvt.Ltd), Potassium acetate (CH_3COOK) (Sisco Research Laboratories Pvt.Ltd), Quercetin (Sisco Research Laboratories Pvt.Ltd),α-amylase (Sisco Research Laboratories Pvt.Ltd) (5U/mg), α-glucosidase (Sisco Research Laboratories Pvt.Ltd) (100U/mg),P-nitro-phenyl-α-D-glucopyranoside (p-NPG) (Sisco Research Laboratories Pvt.Ltd), 3, 5, di-nitro salicylic acid (DNS)(Sisco Research Laboratories Pvt.Ltd), Sodium carbonate (Na_2CO_3) (Avra Synthesis Pvt.Ltd), Acarbose (Sisco Research Laboratories Pvt.Ltd) and all other chemicals of analytical grade were obtained commercially.

7.2 DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

- **Principle:**

The DPPH assay is a widely used method to assess the antioxidant activity of compounds. It involves a stable free radical molecule with a deep purple colour. When an H-donor is introduced, it donates a hydrogen atom to the DPPH radical, reducing it to DPPH-H, with the colour change proportional to the antioxidant content(Baliyan et al., 2022).

- **Material and methods:**

- **Preparation of 1M DPPH solution:**

3.4 mg DPPH was dissolved in 100 ml methanol to produce 1M DPPH solution.

- **Preparation of dilutions of the plant extracts:**

*Artocarpus lakoocha*ethyl acetate (AL-EA) extract 1mg was dissolved in 1ml methanol by the vortex. The solution was filtered through a 0.45µ filter (Millipore). From there, the dilution was prepared with the help of methanol. The IC50 value (50% inhibitory concentration) was measured by nonlinear regression using GraphPad Prism version 10.0 software. IC50 was determined through nonlinear regression of the percentage relative activity f(concentration) plots.

Using the following formula, the DPPH scavenging capacity was determined:

$$\textbf{Scavenging \%} = \left(\frac{\textbf{Absorbance control} - \textbf{Absorbance sample}}{\textbf{Absorbance control}}\right) \times \mathbf{100}$$

- **Methodology:**

We evaluated ALE extract's antioxidant capacity using a DPPH free radical scavenging test that we developed in our lab. In this 96-well microplate, 950 µL of 1M DPPH methanolic solution and an aliquot of 50 µL sample solution at various concentrations (50-100 µg/mL) were mixed. For one hour at 37°C, the reaction mixture was kept in the dark. The removal of the original purple colour showed the extracts' capacity to scavenge free radicals. A UV-visible spectrophotometer was used to measure the absorbance of the reaction mixture at 517 nm(Bhatti et al., 2015). The positive control in this experiment was ascorbic acid.
The above formula was used to obtain the inhibition percentage.

7.3 Nitric Oxide (NO) scavenging assay:

- **Principle:**

The in vitro Nitric Oxide (NO) scavenging assay is a method to evaluate a compound's ability to scavenge nitric oxide, a crucial biological signalling molecule and free radical. It involves generating NO, produced by Sodium nitroprusside (SNP), and measuring nitrite levels as an indicator of NO production. The Griess reaction detects nitrite using sulfanilamide and N-(1-Naphthyl)ethylenediamine dihydrochloride (NED). The assay quantifies the percentage inhibition of NO production and the IC50 value, with controls including known NO scavengers and an SNP solution without any scavenger(Habu & Ibeh, 2015).—ascorbic acid is used as standard or NO scavenger at different concentrations.

- **Material and methods:**

- **Preparation of Sodium nitroprusside (SNP) solution (5 mM):**

14.9mg SNP dissolved in 10ml distilled water to produce 10ml 5mM SNP solution.

- **Preparation of1mg/ml extracts solution:**

AL-EA extract 1mg was dissolved in 1ml of distilled water by the vortex. The solution was filtered through a 0.45µ filter (Millipore).

- **Methodology:**

In the 96-well plate, add 60 µL of the test compounds or extracts at different concentrations. Add 60 µL of the SNP solution (5 mM) to each well.Incubate the plate at room temperature for 2-3 hours under light conditions to generate NO.After incubation, add 120 µL of the Griess reagent to each well.Incubate the plate for 10-15 minutes at room temperature to allow the development of colour.Measure the absorbance of the wells at 540 nm using a microplate reader(Gangwar et al., 2014).

Using the following formula, the NO scavenging capacity was determined:

$$\textbf{Scavenging \%} = \left(\frac{\textbf{Absorbancecontrol} - \textbf{Absorbancesample}}{\textbf{Absorbancecontrol}}\right) \times \mathbf{100}$$

7.4 Determination of Total Phenolic Content (TPC):

- **Principle:**

The reduction of the Folin–Ciocalteu reagent (FCR) in the presence of phenolics is the basis of the widely used Folin–Ciocalteu (F–C) test for phenolic analysis. Molybdenum-tungsten blue is produced as a consequence, and its concentration increases linearly with the concentration of phenolics in the reaction media. It is detected spectrophotometrically at 760 nm. By lowering precipitate formation and raising the assay's reactivity, lithium sulfate is added to the reagent, which improves the FCR. Fruits and vegetables may have their total phenolic content determined using the popular and easy-to-use F-C test. The method calls for adding 1.25 millilitres of a sodium carbonate solution (7%) to a test tube after it has been filled with 0.5 millilitres of deionized water, 0.125 millilitres of diluted fruit extracts, and 0.125 millilitres of FCR and allowing it to react for six minutes (Malta & Liu, 2014).

To ascertain the amount of phenol that is contained inside a sample. Because phenols may scavenge because of their hydroxyl group, the antioxidant activity of a particular plant is also characterized. Gallic acid was the standard for this procedure, and the oxidizing agent was the Folin-Ciocalteau reagent. In terms of gallic acid equivalent (mg GAE/g), the TPC was estimated(Dai & Mumper, 2010).

- **Material and Methods:**

Bioactive substances that provide health advantages are called phytochemicals, especially phenolics in plants. These substances have various biological effects, including antioxidant activity, and may be found in edible and inedible plant components. They can scavenge because of their redox characteristics, enabling them to function as hydrogen donors, reducing agents, and singlet oxygen quenchers. In addition, natural antioxidants have antiviral, antibacterial, anti-inflammatory, antiallergic, antithrombotic, and vasodilatory properties. Researchers and food makers are becoming more interested in the potential of these phytochemicals for maintaining health and protecting against cancer and coronary heart disease(Babbar et al., 2011).

- **Preparation of Folin cio-calteau phenol reagent:**

For 1:10 dilution in 10 ml total volume: 1 part Folin-Ciocalteu reagent = 1 ml, 9 parts water = 9 ml.

- **Preparation of standard Gallic acid dilutions:**

Gallic acid with a 95% purity level was used to create standard dilutions. A solution containing 1 mg/ml was produced, and then six dilutions of 1000, 500, 250, 125, 62.5, and 31.25 µg/ml were serially diluted.

- **Preparation of 6% $NaHCO_3$ solution:**

For 10 ml, 0.6 g of $NaHCO_3$is needed.

- **Preparation of 1mg/ml extract solution:**

AL-EA extract 1mg was dissolved in 1ml of distilled water by the vortex. The solution was filtered through a 0.45µ filter (Millipore).

- **Methodology:**

A 0.1 mL aliquot of each extract (1 mg/mL) and 0.75 mL of 10-fold diluted Folin-Ciocalteu reagent were mixed in a 96-well plate. After letting the mixture sit at room temperature for five minutes, 0.75 mL of 6% sodium carbonate was added. Following a 90-minute procedure, the absorbance at 725 nm was determined. The standard calibration curve was developed using gallic acid. Per gram of dry weight, the total phenolics were expressed in milligrams of gallic acid equivalent. To generate the negative control, 0.1 mL of distilled water was substituted for the extract (Bhatti et al., 2015).
An equation derived from the standard gallic acid curve was used to calculate the gallic acid content in a sample.

The formula is **Y= mx+c**

Y- absorbance

x- gallic acid concentration µ/ml

m – slope

c- Intersection

7.5Determination of Total flavonoid content(TFC):

- **Principle:**

Plant extracts were tested for TFC using the aluminium chloride colourimetry technique. This approach uses Al (III) as a complexing agent, forming Al(III)-flavonoid chelates. Depending on the pH level and the circumstances of the experiment, flavonoids have a considerable propensity to bind metal ions, usually at a 1:1 ratio(Shraim et al., 2021).
Quercetin serves as a standard to ascertain the amount of flavonoids contained in a sample. In terms of quercetin equivalents (mg/QE/g), the total flavonoid content was assessed.

- **Material and Methods**

- **Preparation of Quercetin solution:**

1mg was dissolved in 1ml methanol by the vortex, and six dilutions of 1000, 500, 250, 125, and 62.5µg/ml were serially diluted.

- **Preparation of 10 % $AlCl_3$ solution:**

1.67g $AlCl_3$ dissolved in 10ml distilled water.

- **Preparation of 1M CH_3COOK solutions:**

0.9841g CH_3COOK dissolved in 10ml distilled water.

- **Preparation of 1mg/ml extract solution:**

AL-EA extract 1mg was dissolved in 1ml methanol by the vortex. The solution was filtered through a 0.45µ filter (Millipore).

- **Methodology:**

1.5 mL of methanol and an aliquot of 0.5 mL of various extracts (1 mg/mL) were mixed on a 96-well plate. The following ingredients added were 2.8 mL of distilled water, 0.1 mL of potassium acetate (1 M), and 0.1 mL of 10% aluminium chloride. The reaction mixture was allowed to come to room temperature for half an hour. We measured the absorbance of the reaction mixture at 415 nm. Rutin was used as the standard to construct the calibration curve. For each gram of dry weight, the flavonoid concentration was expressed as milligrams of quercetin equivalent.

The formula is as follows: **Y= mx+c**

Y- absorbance

x- Quercetin concentration µ/ml

m – slope

c- Intersection

7.6Evaluation of in vitro antidiabetic potential

7.6.1 α-amylase:

- **Principle:**

α-amylase enzymes catalyze the hydrolysis of α-1,4-glycosidic linkages in starch and similar polysaccharides. One necessary biochemical approach to evaluate the activity of these enzymes is the alpha-amylase in vitro test. Monitoring when substrate vanishes or when products emerge under carefully regulated circumstances quantifies this process. Typical substrates include specific synthetic substrates, starch, amylose, and amylopectin. The assay's sensitivity and specificity may be impacted by the substrate used. The Dinitrosalicylic Acid (DNS) Method, the Iodine-Starch Reaction, the Chromatometric Substrate Method, and the Turbidimetric Method are examples of colourimetric techniques. Through the conversion of insoluble starch into soluble products, these techniques assess the decrease in turbidity, the reduction in sugars generated by the enzyme, and the intensity of the blue colour(Souza, 2010).

- **Preparation of 100mM phosphate buffer pH 6.8:**

1.7798g $Na_2HPO4.2H_2O$, 1.3609g KH_2PO_4 were dissolved in 100ml water and maintain pH 6.8.

- **Preparation of α-amylase (2U/ml) solution:**

4mg α-amylase dissolved in 8ml 100mM phosphate buffer pH 6.8.

- **Preparation of DNS solution:**

7ml of 0.5N NaOH solution, 3g sodium potassium tartrate, and 0.1g of DNS, gradually add into 10ml distilled water.

- **Preparation of 1mg/ml extract solution:**

AL-EA extract 1mg was dissolved in 1ml methanol by the vortex. The solution was filtered through a 0.45µ filter (Millipore).

Methodology:

The conventional procedure was followed with a bit of modification to determine the extract's and fractions' alpha-amylase inhibitory activity. A 96-well plate was preincubated for 20 minutes at 37°C with a reaction mixture that included 50 µl of phosphate buffer (100 mM, pH = 6.8), 10 µl of α-amylase (2 U/ml), and 20 µl of extract and fractions at various concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml). 30 minutes at 37°C were spent incubating the mixture after 20µl of 1% soluble starch (100 mM phosphate buffer, pH 6.8) was added as a substrate. After mixing, the mixture's absorbance was measured by boiling it for 10 minutes with 100µl of DNS colour reagent added. Using a Multiplate Reader, the measurement was made at 540 nm.The standard was acarbose at a range of concentrations (1 mg/ml). The findings were presented as a percentage of inhibition(Telagari & Hullatti, 2015).The results were expressed as percentage inhibition, which was calculated using the formula-

Inhibitory activity (%) = $(1-A_S/A_C) \times 100$

Where,
A_S = absorbance in the presence of test substance
A_C = absorbance of control

7.6.2 α-glucosidase:

- **Principle:**

α-glucosidase in vitro assays are an essential biochemical method for determining the activity of α-glucosidase enzymes, and they are especially significant in the fields of food science, medication development, and diabetes research. The release of glucose or a chromogenic/fluorogenic group from a particular substrate under regulated circumstances is measured to quantify the activity. p-Nitrophenyl-α-D-glucopyranoside (pNPG), 4-Methylumbelliferyl-α-D-glucopyranoside, maltose, and other naturally occurring oligosaccharides are examples of common substrates. Among the measurement techniques are the glucose oxidase-peroxidase system for natural substrates, spectrophotometric measurement of p-nitrophenol using pNPG, and fluorometric quantification of 4-methylumbelliferone using 4-methylumbelliferyl-α-D-glucopyranoside(Daou et al., 2022).

- **Preparation of 100mM phosphate buffer pH 6.8:**

1.7798g $Na_2HPO4.2H_2O$, 1.3609g KH_2PO_4 were dissolved in 100ml water and maintain pH 6.8.

- **Preparation of α-glucosidase (1U/ml) solution:**

4 mg glucosidase dissolved in 4ml 100mM phosphate buffer pH 6.8.

- **Preparation of 0.1M Na_2CO_3 solution:**

0.106g Na2CO3 solution dissolved in 10ml 100mM phosphate buffer pH 6.8.

- **Preparation of 1mg/ml extract solution:**

AL-EA extract 1mg was dissolved in 1ml methanol by the vortex. The solution was filtered through a 0.45µ filter (Millipore).

- **Methodology:**

The standard protocol was used with minor adjustments to ascertain the extract and fractions' α-glucosidase inhibitory activity. A 96-well plate was preincubated for 15 minutes at 37°C with a reaction mixture that included 50µl phosphate buffer (100 mM, pH = 6. 8), 10µl α-glucosidase (1 U/ml), and 20 µl extract and fractions at various concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml). After adding 20 µl P-NPG (5 mM) as a substrate, the mixture was incubated for a further 20 minutes at 37°C. By adding 50 µl Na_2CO_3 (0.1 M), the process was halted. The Multiplate Reader was used to measure the absorbance of the emitted p-nitrophenol at 405 nm. As a standard, different doses of acarbose (0.1–0.5 mg/ml) were used. It was established in parallel without a test material (Telagari & Hullatti, 2015). The formula was used to compute the % inhibition, which was used to express the findings-

Inhibitory activity (%) = $(1-A_S/A_C) \times 100$

Where,
A_S = The absorbance in the presence of the test substance
A_C= Theabsorbance of control

7.7*In vivo* Study:

7.7.1 Material and Methods:

7.7.1.1 Animals

The optimal study employed 7-8 weeks ofWistar albino rats of either sex that weighed 140-160 g. Rats were maintained in a conventional laboratory setting with 12-hour light/dark cycles, a temperature of 25-28 °C, and a relative humidity of 55-60%. They were fed a high-fat diet, which contained30% fat, 20-30% protein, 35% carbohydrate, and 25% fructose, and they were given access to water as needed. Prior to the trial, the animals spent a week becoming used to the lab environment.

7.7.1.2 Drugs and chemicals:

Streptozotocin was purchased from Sisco Research Laboratories Pvt Ltd. India and Metformin (Sisco Research Laboratories), Trichloroacetic acid (TCA) (Sisco Research Laboratories Pvt.Ltd), Thiobarbituric acid (TBAS) (Sisco Research Laboratories Pvt.Ltd), Phenazonium methosulfate (PMS), 5,5'-dithio bis-2-nitro benzoic acid (DTNB), Nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH) from SISCO Research Labomiceory,Mumbai, India; Potassium dihydrogen phosphate (Sisco Research Laboratories Pvt.Ltd), Sodium Phosphate dibasic dihydrate (Avra Synthesis Pvt.Ltd), Potassium dichromate (Sisco Research Laboratories Pvt.Ltd), Acetic acid from Ranbaxy, Mumbai; Citric acid (Sisco Research Laboratories Pvt.Ltd), Sodium citrate (Sisco Research Laboratories Pvt.Ltd), Sodium dodecyl sulfate (SDS) (Avra Synthesis Pvt.Ltd), Malondialdehyde (MDA) (Sisco Research Laboratories Pvt.Ltd) and all the other reagents kits used were from Arkray.

7.7.1.3 Induction of diabetes in rats:

A single intraperitoneal injection of 40 mg/kg streptozotocin, prepared in 0.1 mol citrate buffer pH 4.4, was administered to rats. Parallel to the diabetic rats, age-matched control rats were given citrate buffer. Blood samples were taken two days after the STZ injection, and the Counter Plus and diagnostic kit technique (Arkray) was used to determine the plasma glucose levels. In the current investigation, rats with plasma glucose levels of more than 250 mg/dl after four weeks were chosen.

7.7.1.4 Treatment schedule:

The rats were continued with high-fat diets throughout the study. The therapy was given to the animals for 28 days after they were split up into six groups (n = 6).

- **<u>Experimental groups and dose schedule:</u>**

- **Group I (NC):** Normal control group (Rats on normal diet).
- **Group II (DC):** Disease control group + Streptozotocin (STZ) (Rats fed with HFD daily for 28 days).
- **Group III (Low):** HFD rats + STZ +*Artocarpus lakoocha* extract (ALE) low dose (100 mg/kg body weight) orally daily for two weeks.

- **Group IV (Medium):** HFD rats + STZ +ALE high dose (200 mg/kg body weight) orally daily for two weeks.
- **Group V (High):** HFD rats + STZ +ALE high dose (400 mg/kg body weight) orally daily for two weeks.
- **Group VI (STD):** Standard control group (HFD rats + STZ + Metformin 250 mg/kg body weight) orally daily for two weeks.

7.7.2 Estimation of fasting blood glucose (FBG) level

Using a one-touch glucometer, the FBG level was tested on the 0th, 7th, 14th, 21st prime, and 28th day. All rats in each group had their blood drawn by heart puncture for the purpose of estimating the serum lipid profile (LDL, VLDL, TC, TG) and serum biochemical parameters (SGOT or AST, SGPT or ALT, De-ritis ratio) after the twenty-first hour following the last dosage and after eighteen hours of fasting. After that, the animals were killed so that liver tissue could be collected and various endogenous antioxidant parameters could be examined.

7.7.3 Body weight:

On days 0, 7, 14, 21, and 28 of each group, the body weight of the rats was measured. To ensure accuracy, a conventional digital weight balance was used to measure the weight.

7.7.4 Serum Liver Function Test

Numerous serum biochemical parameters, such as serum glutamic pyruvic transaminase (SGPT) or alanine transaminase (ALT), and serum glutamic oxaloacetic transaminase (SGOT) or aspartate aminotransferase (AST), were measured in the collected blood. Commercially accessible kits from Span Diagnostics Ltd., India, were used for all of the analyses.

7.7.5 Serum lipid profiles

Using commercial kits from Span Diagnostics Ltd. India, serum lipid profiles such as total cholesterol, HDL cholesterol, triglycerides, and LDL cholesterol were measured in STZ-induced diabetic rats.

7.7.6 Sacrifice and collection of livers

The deaths occurred during the conclusion period for each group. The rats were fasted for eight hours before being humanely killed with halothane at the end of the intervention period. Their livers were removed and cleaned with regular saline. Each liver had a piece of around 5 mm removed, which was then preserved in 10% neutral buffered formalin for histology. Five millilitres of sodium phosphate buffer (pH 7.5; 50 mM) containing 10% Triton X100 was used to homogenize 0.5 grams of each heart that was removed. After homogenization, the tissue samples were centrifuged for 10 minutes at 4°C at 15,000 rpm. After being decanted into Eppendorf tubes with labels, the supernatants were kept at -20°C until further analysis could be performed.

7.7.7 Histopathological Studies:

Hematoxylin and eosin (H&E) staining, oil-o-red staining, and standard paraffin tissue processing were performed on liver tissues that had been preserved in 10% normal saline. A Digital Bright-field Microscope (OMAX, USA) was used to examine the slides, and digital photomicrographs were produced.

7.7.8 Estimation of Liver Antioxidant Stress

Catalase activity, lipid peroxidation (MDA), and reduced glutathione (GSH) levels were among the oxidative stress indicators measured in tissue supernatants.

7.7.8.1 Reduced glutathione (GSH) level

The GSH level was ascertained using Ellman's method. To summarize, 100 µL of the tissue supernatant was deproteinized with 300 µL of 10% TCA, and the resulting mixture was centrifuged at 3500 rpm for five minutes. After that, a 96-well plate was pipetted with 200 µL of the deproteinized samples and 50 µL of Ellman's solution, and it was incubated for five minutes. At 415 nm, the absorbance was measured. Utilizing a GSH standard curve, the GSH level was determined(Gl, 1959).

7.7.8.2 Catalase activity

The technique that was previously developed was used to determine the catalase activity. In summary, 340 µL of 50 mM sodium phosphate buffer (pH 7.0) was combined with 100 µL of the supernatants. After that, the mixture was mixed with 150 µL of 2 M H_2O_2. For three minutes, absorbance was measured at 240 nm at one-minute intervals(Aebi, 1984).

7.7.8.3 Lipid peroxidation levels

Using the previously described protocol, the amounts of thiobarbituric acid reactive substances (TBARS) were quantified and expressed as malondialdehyde (MDA). To summarize, a reaction mixture including 100 µL of the supernatant, 100 µL of 8.1% SDS solution, 375 µL of 20% acetic acid, and 1 mL of 0.25% thiobarbituric acid (TBA) was prepared. 200 µL of the heated liquid was pipetted onto a 96-well plate, and the absorbance was measured at 532 nm after the liquid had boiled for an hour. The MDA values were extrapolated using an MDA standard curve(Chowdhury & Soulsby, 2002).

7.7.9 Statistical Analysis

Every result is shown as mean ±SEM. Using Graph Pad Prism 10.0 software (Graph Pad Software, USA), one-way analysis of variance (ANOVA) and post hoc Dunnett's test were used to assess the data for statistical significance. P values were deemed statistically significant when they were less than 0.05.

RESULT

8.1 The physicochemical evaluation of plants involves assessing both physical and chemical properties to understand their composition, quality, and potential applications. The below results evaluate the potential physiochemical parameters of *Artocarpus lakoocha* (AL).

Table 1:Physicochemical evaluation of ALE

Standardization parameters	**Value**
Moisture content	10.667±0.5774
Ash value:-	
Total ash value	16.667±0.5774
Acid insoluble ash value	11±1
Water soluble ash value	9.333±0.471

Extractive value (%w/w):-	
Solvent	**Extractive value**
Ethyl acetate	12.6±0

Values are mean ± SD, (n= 3)

8.2

Table 2:Preliminary phytochemical screening of ALE

SI.NO		**PLANT EXTRACT**	**RESULT**
1.	Alkaloids	Mayer's reagent	Positive
		Hager's reagent	Positive
		Wagner's reagent	Positive
2.	Phenol phenolic compounds	$FeCl_3$	Positive
		Lead acetate test	Negative
3.	Saponin	Frothing test	Positive
4.	Carbohydrate	Molisch test	Positive
		Fehling's solution	Positive

		Benedict's test	Positive
5.	Protein	Millon's test	Positive
		Xantho Protein test	Positive
6.	Glycosides	Bontrager's test	Negative
		Kellar Kiliani test	Positive
7.	Flavonoids	Lead acetate solution test	Negative
		Shinoda test	Positive
		H_2SO_4 test	Positive
8.	Triterpenoid	Salkowski's test	Positive
9.	Tannins		Positive
10.	Phlabotanin		Positive
11.	Anthraquinones		Negative
12.	Quinones		Negative
13.	Resins		Negative

8.3 Observation of absorbance in Total Phenolic Content (TPC) estimation

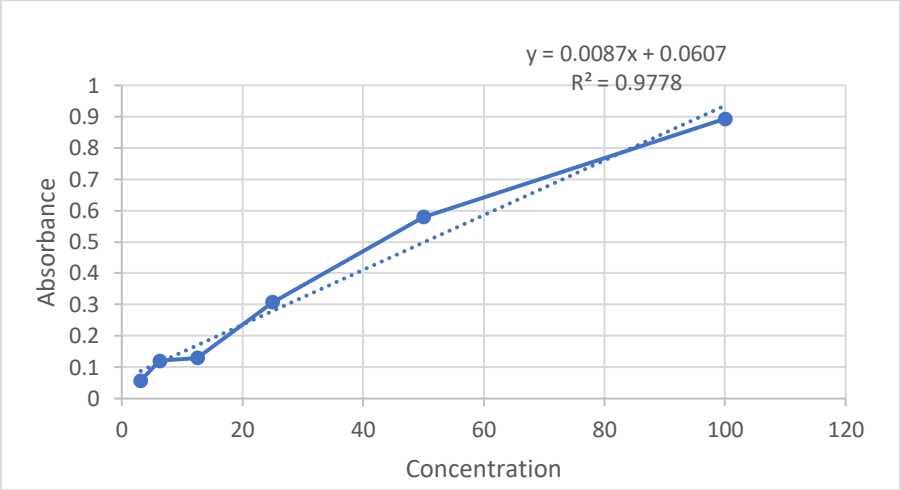
The absorbance of the Standard and test solution were represented in table no.3 The total phenolic content in terms of mg Gallic Acid Equivalent(GAE)of Ethylether extract was found to be 96.35 mg/g. These results suggest that the higher presence of phenolic components was responsible for the levels of antioxidant activity. The standard curve for gallic acid is shown in the figure 12.

Table3 :

Sl No.		**Concentration(µg/ml)**	**Absorbance**
1	Standard (Gallic acid)	31.25	0.055
2		625	0.012
3		125	0.012
4		250	0.030
5		500	0.057
		1000	0.893
1	Sample (Ethyl ether extract)	1000	0.899

Values are mean ± SD, (n= 3)

Figure12: Standard curve of Gallic acid



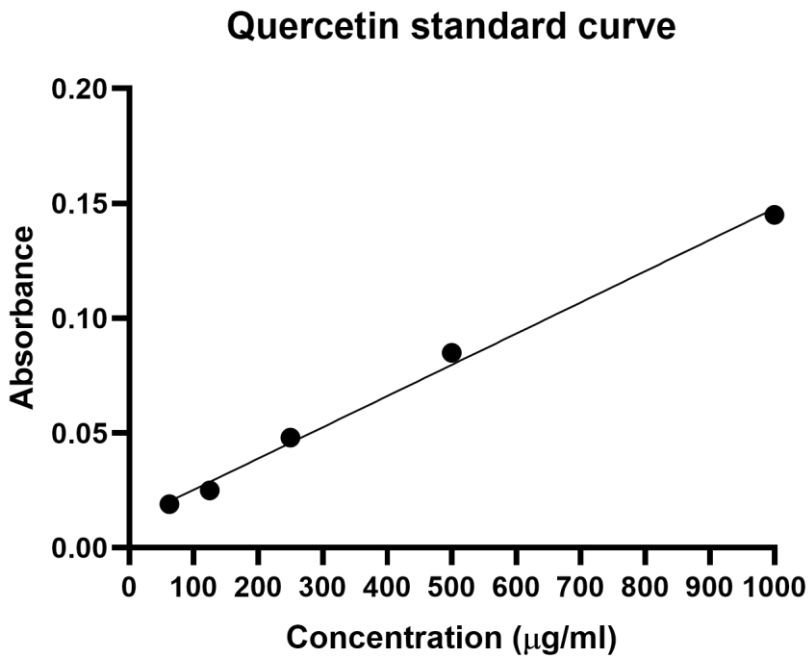
8.4 Observation of absorbance in total flavonoid content (TFC) estimation

The content of the flavonoid compound in the Ethyl ether extract of *Artocarpus lakoocha* barkwas measured by aluminum chloride reagent in terms of quercetin equivalent. It was found to be 435.956 mg/g.The absorbance of the Standard and test solution were represented in Table no. 4. It is well known that flavonoids have significant antioxidant activity and have a positive impact on human nutrition and health. The ethyl acetate extract contains a substantial quantity of flavonoids, which may contribute considerably to the plant's antioxidant activity.The Standard curve of Quercetin is shown in Figure 13.

Table 4:

Sl No.		**Concentration(µg/ml)**	**Absorbance**
1	STANDARD(QUERCETIN)	1000	0.145
2		500	0.085
3		250	0.048
4		125	0.025
5		62.5	0.019
1	SAMPLE (Ethyl ether extract)	1000	0.071

Figure13: Standard curve of Quercetin



8.5 Characterization of tincture

The different characteristics of the prepared tincture are shown in table 5, 6 and 7. The pH of the prepared tincture to varying strengths of alcohol is represented in table 5. The study shows that greater alcohol concentration in the tincture increases the acidity of the tincture, while storage for a more extended period also slightly increases acidic properties. The solid content of the tincture also decreases with time, as seen from table 6, while greater alcohol concentration also imparts diminished solid content. Finally, as seen intable 7, the specific gravity of the prepared tinctures does not vary substantially with time.

Table 5: pH of the tincture

Alcohol Strength	**pH**		
	7 Days	**14 Days**	**21 Days**
40%	6.05	5.85	5.73
50%	5.93	5.81	5.64

60%	5.84	5.64	5.60
70%	5.73	5.59	5.54
80%	5.71	5.63	5.59
90%	5.39	5.56	5.58
Absolute(100%)	5.34	5.24	5.07

Table 6:TotalSolidContentofTincture

Alcohol Strength	**7 Days**	**14 Days**	**21 Days**
40%	19.09	17.2	14
50%	17.18	15.47	12.83
60%	16.95	15.38	12.5
70%	16.69	14.81	10.33
80%	15.96	13.57	10.71
90%	12.13	13.28	10.16
Absolute(100%)	9.93	7.17	5.53

Table 7: Specific Gravity of Tincture

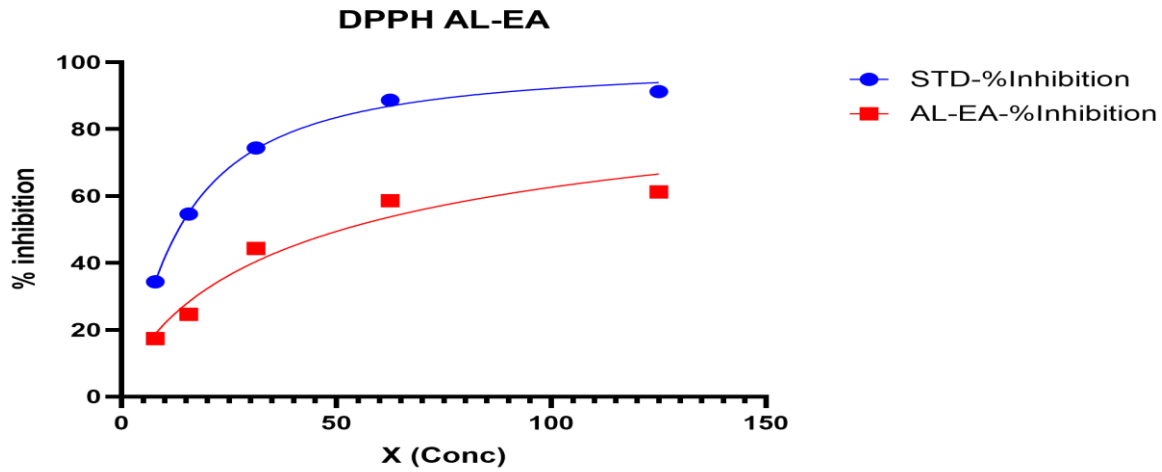
Alcohol Strength	7 Days	14 Days	21 Days
40%	0.952	0.950	0.954
50%	0.932	0.941	0.946
60%	0.900	0.921	0.949
70%	0.887	0.895	0.902
80%	0.865	0.899	0.912
90%	0.841	0.900	0.929
Absolute(100%)	0.797	0.799	0.801

8.6 In vitro

8.6.1 %Inhibition of DPPH radicals:

The ability of DPPH to be reduced was evaluated by measuring the decrease in its absorbance at 517 nm, which is caused by antioxidants. When DPPH free radicals were present in large quantities, the extract demonstrated its highest capacity to donate hydrogen. The extract has an IC50 value of 51.52µg/ml, indicating antioxidant activity. On the other hand, the DPPH radical showed an IC50 value of 13.26 µg/ml for the well-known antioxidant ascorbic acid. When compared to ascorbic acid, A. lakoocha exhibited substantial scavenging effects with increasing concentration. Figure 14 below displays the findings of the DPPH scavenging activity and shows the assay results for the DPPH radical scavenging of the test and reference samples.

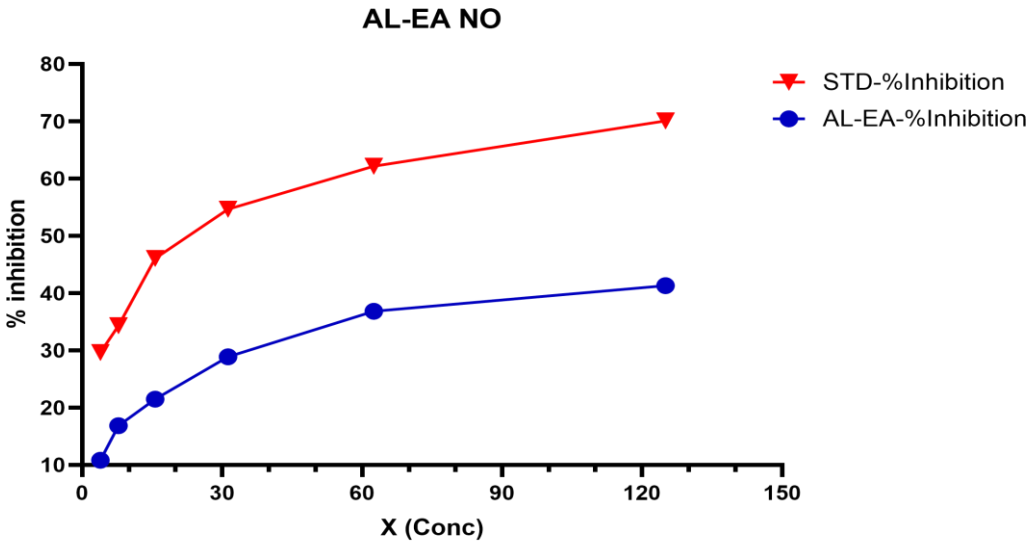
Figure14:DPPH radical scavenging assay



8.6.2% Inhibition of NO radicals:

Nitric oxide radicals produced by sodium nitroprusside are the source of the NO scavenging assay, which is based on the competition between a test chemical and oxygen. Following treatment with the Griess reagent, a drop in absorbance 540 nm is indicative of a lower nitrite content, which in turn indicates the compound's scavenging abilities. Compounds with possible anti-inflammatory and antioxidant qualities may be screened for using this method.The extract has an IC50 value of 223 µg/ml, indicating antioxidant activity. On the other hand, the IC50 value of Ascorbic acid, a well-known antioxidant, on NO radical was 23.06 µg/ml. When compared to ascorbic acid, A. lakoocha exhibited substantial scavenging effects with increasing concentration. Figure 15 below displays the NO scavenging activity findings for the test and reference samples in the NO radical scavenging experiment.

Figure15: NO radical scavenging assay

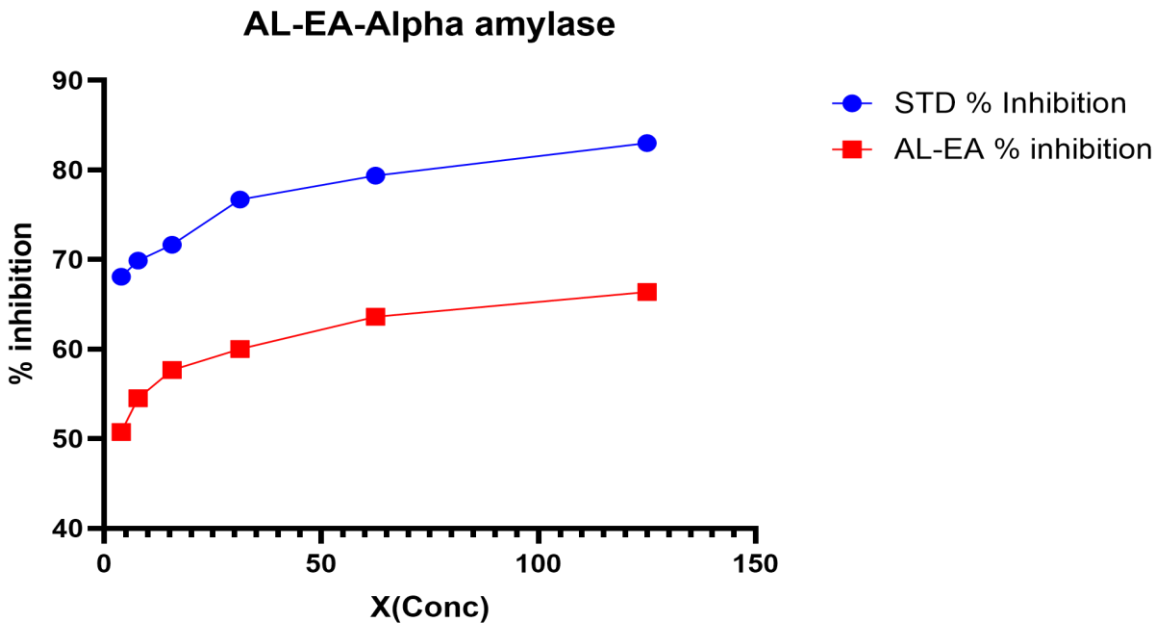


8.7 Determination of *in-vitro*Antidiabetic Activity:

8.7.1α-amylase inhibition assay:

One of the enzymes that breaks down the α-1,4 glycosidic bonds in starch to release glucose and maltose is called α-amylase. α-amylase hydrolyzes complex polysaccharides into oligosaccharides and disaccharides, which are then further hydrolyzed into monosaccharides by α-glucosidase. The α amylase inhibitory test was used to assess the in vitro antidiabetic efficacy. A. lakoocha's ethyl acetate extract inhibits this enzyme in several ways. As a standard, acarbose has an intense enzyme inhibitory action. Acarbose and ethyl acetate extract were determined to have IC50 values of 0.1985 µg/ml and 3.131 µg/ml, respectively. The graph of Acarbose and the test sample's α-amylase inhibition experiment is shown in Figure 16.

Figure 16: Graph of α-amylase inhibition assay of Acarbose and ALE sample

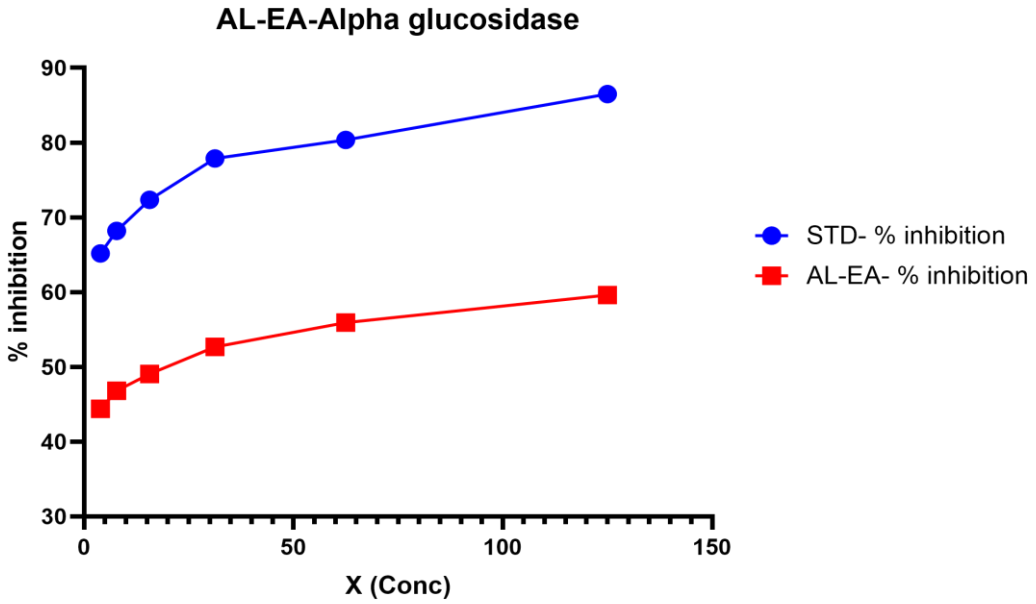


8.7.2α-glucosidase inhibition assay:

α-glucosidase inhibitory agents are effective in controlling postprandial hyperglycemia in frank diabetes patients by reversibly inhibiting the digestion of disaccharides and converting them into monosaccharides, thereby lowering blood glucose levels. Many natural products with α-glucosidase inhibitory activity have shown significant reductions.The in vitro antidiabetic activity was evaluated by using the α-glucosidase inhibitory assay. The ethyl acetate extract of *A.lakoocha*produces some inhibitory effects on this enzyme. Acarbose was used as a standard, which shows marked inhibitory effect of the enzyme. The IC_{50} of Acarbose and Ethyl acetate extract was calculated and found to be 0.6816µg/ml and 16.03

µg/ml, respectively.Figure 17represents the graph of the α-glucosidase inhibition assay of Acarbose and test sample.

Figure 17: Graph of α-glucosidase inhibition assay of Acarbose and AL-EA sample

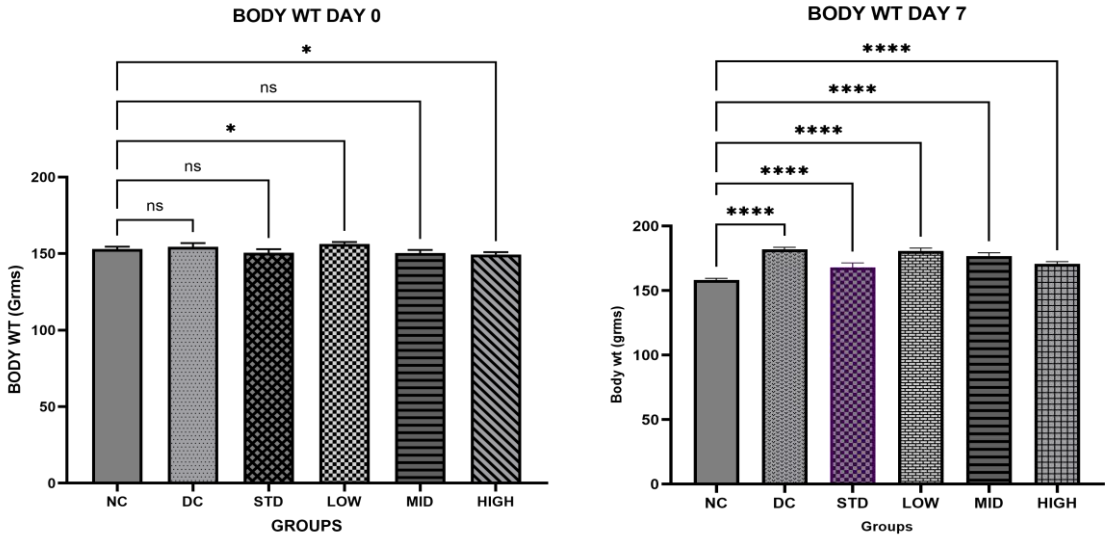


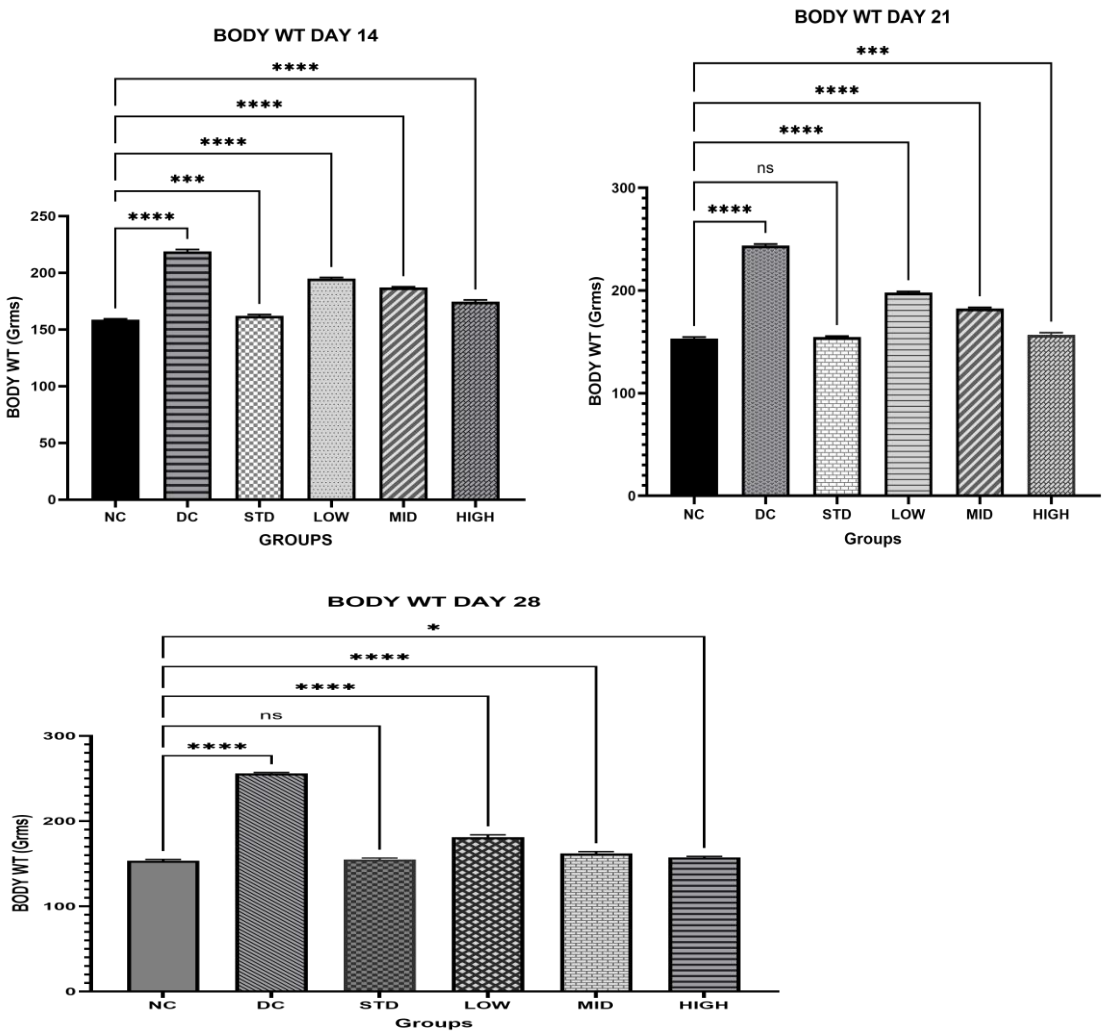
8.8 In vivo

8.8.1Body weight

Compared to the normal control group, the diabetes control group's end body weights were considerably ($p < 0.05$) lower. When ALE at dosages of 100, 200, and 400 mg/kg was administered, body weight improved dramatically ($p<0.05$) in comparison to the diabetes control group.

- **Effect of ALE on body weight**

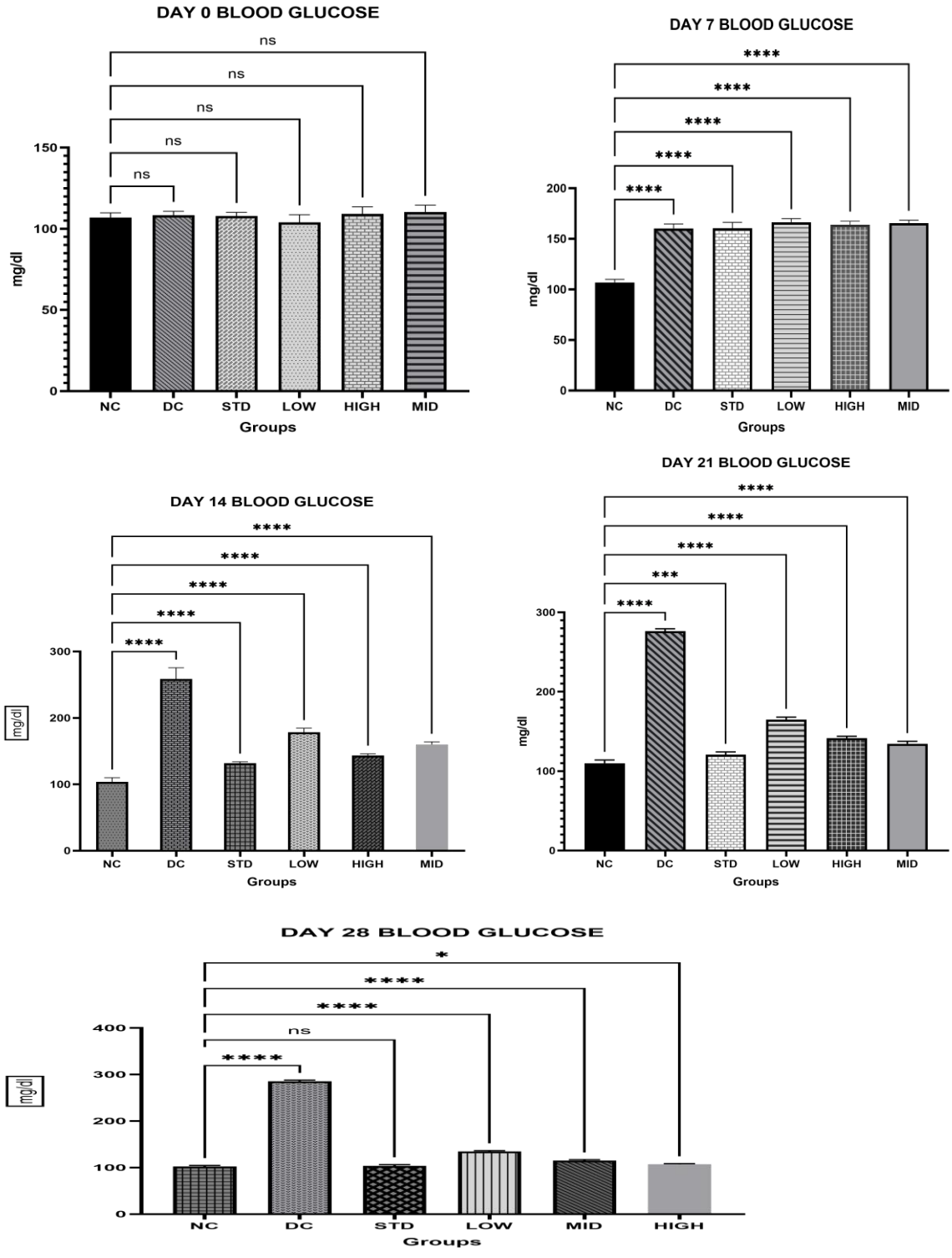




8.8.2Fasting blood glucose (FBG) level

Rats given STZ to induce diabetes had considerably ($p < 0.05$) higher FBG levels than the healthy control group. When ALE was administered to diabetic rats at dosages of 100, 200, and 400 mg/kg, the FBG level was dramatically ($p < 0.05$) lowered towards normal in comparison to the diabetic control group.

- **Effect of ALE on fasting blood glucose(mg/dl)**

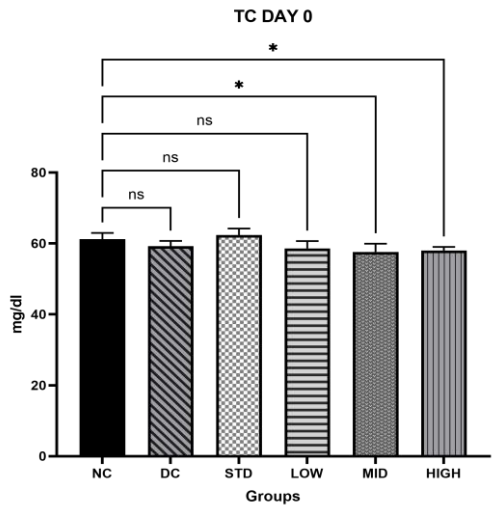


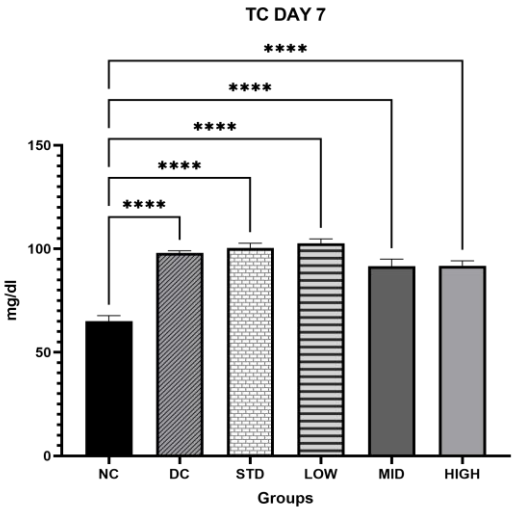
8.8.3Estimation of serum lipid profiles:

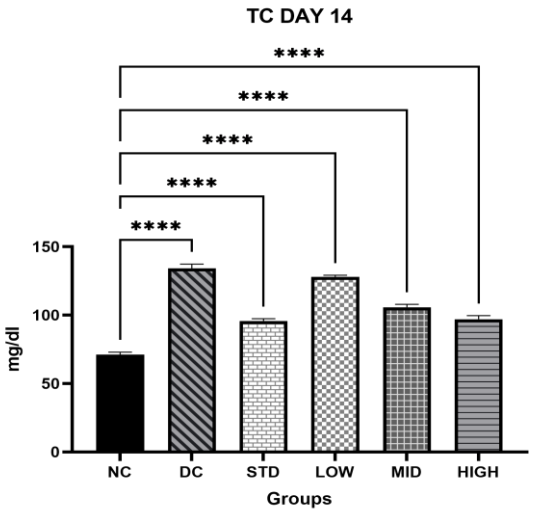
In comparison to the normal control group, the STZ-induced diabetic rats showed considerably ($p < 0.05$) raised serum lipid profiles such as TC, LDL, and VLDL and significantly ($p < 0.05$) reduced HDL levels. When compared to the diabetic control group,

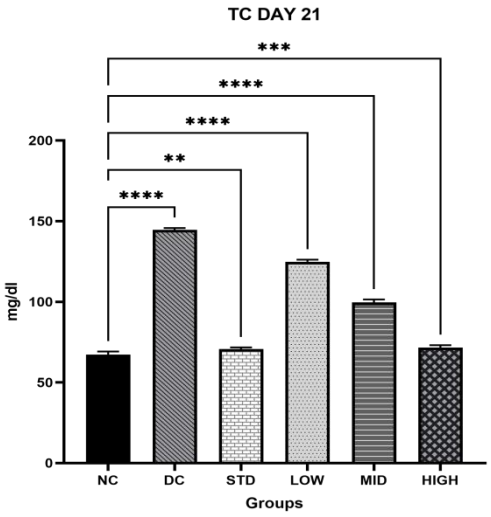
treatment with ALE at the dosages of 100, 200, and 400 mg/kg considerably ($p < 0.05$) decreased the levels of TC, LDL, and VLDL and significantly (p - 0.05) improved the level of HDL.

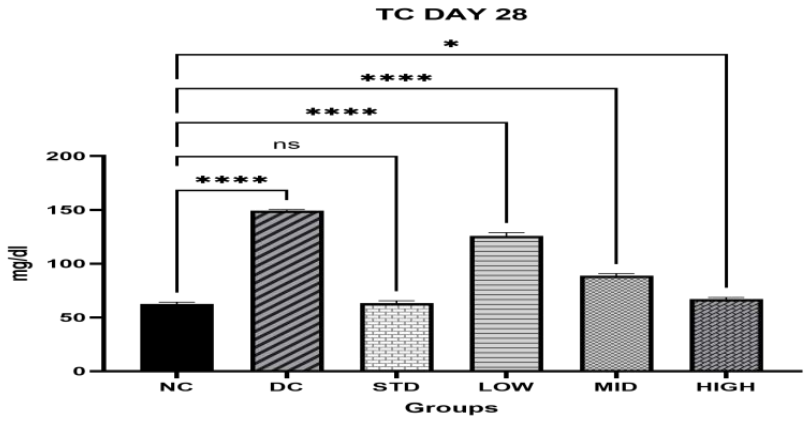
- **Effect of ALE on TC:**



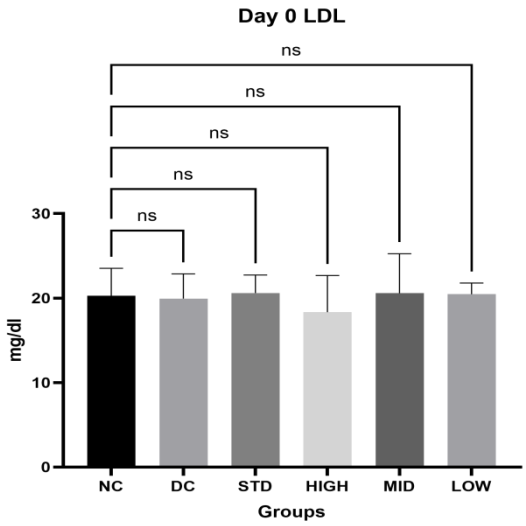


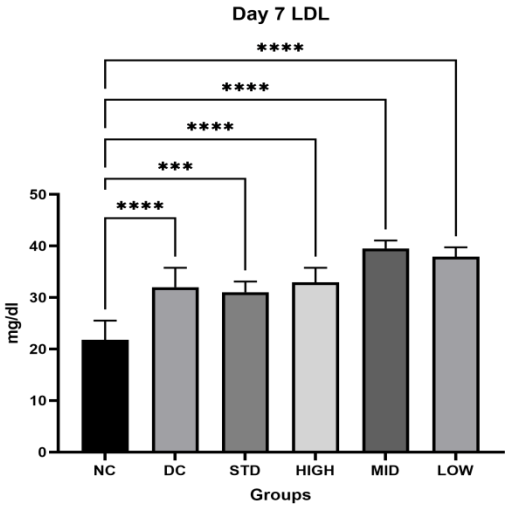


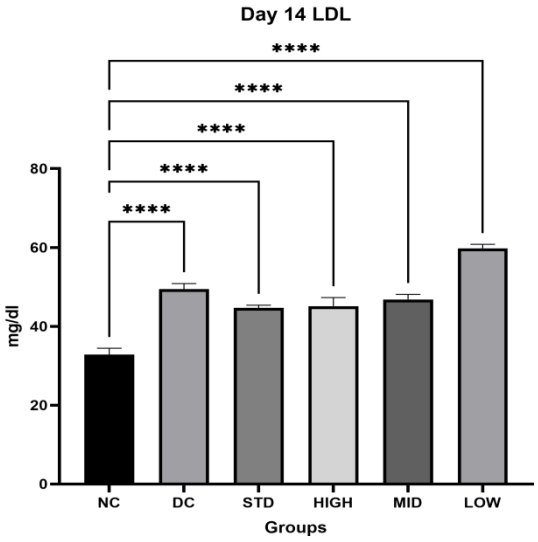


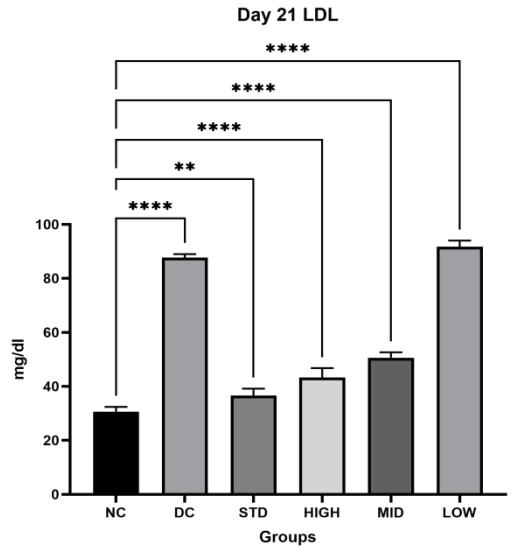


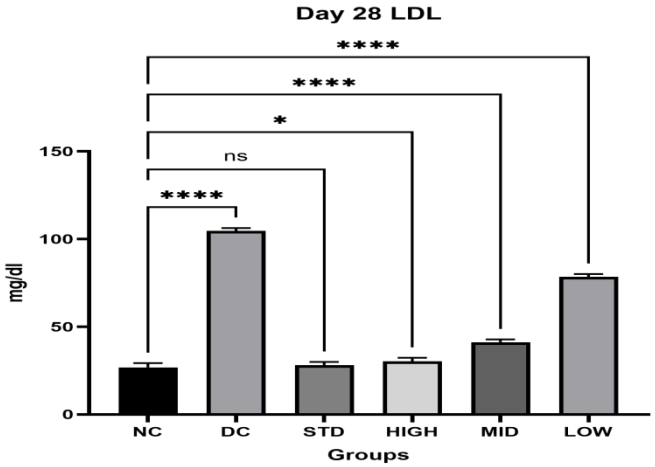
➢ Effect of ALE on LDL:



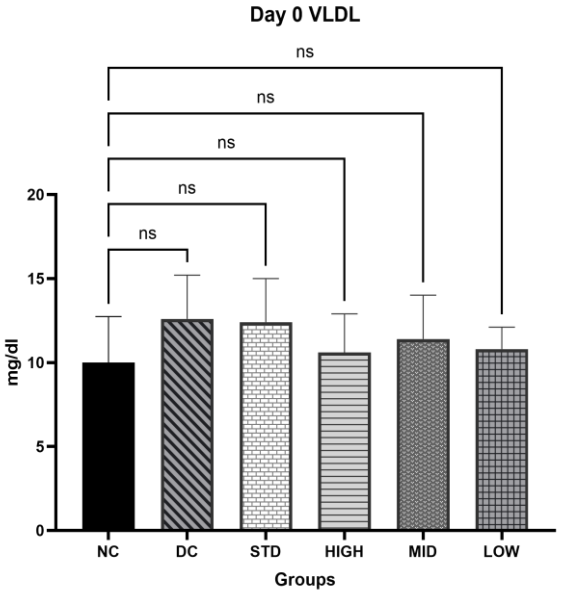


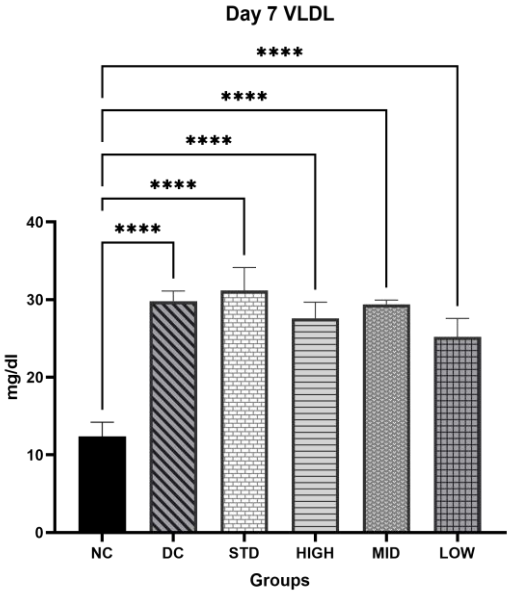


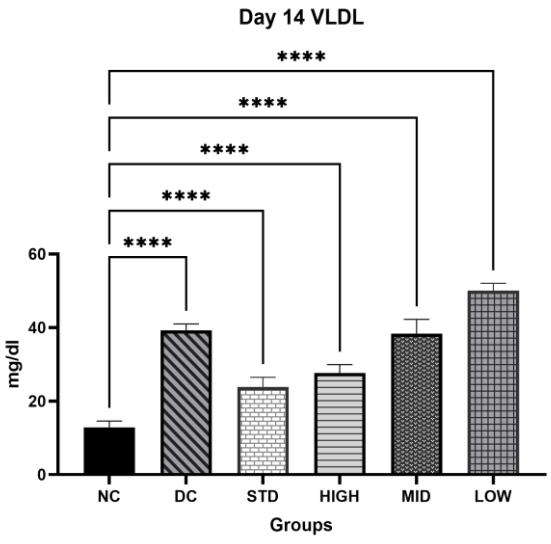


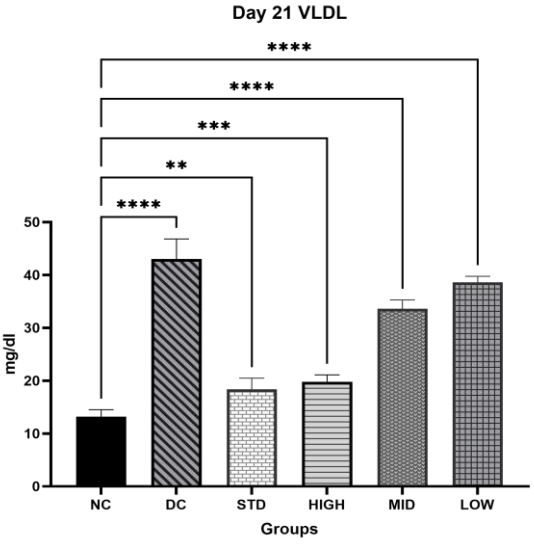


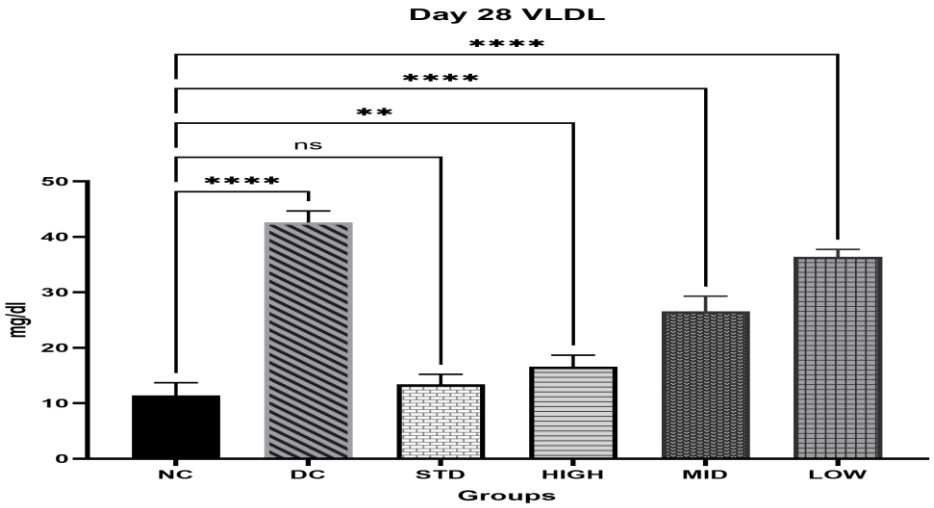
- **Effect of ALE on VLDL:**



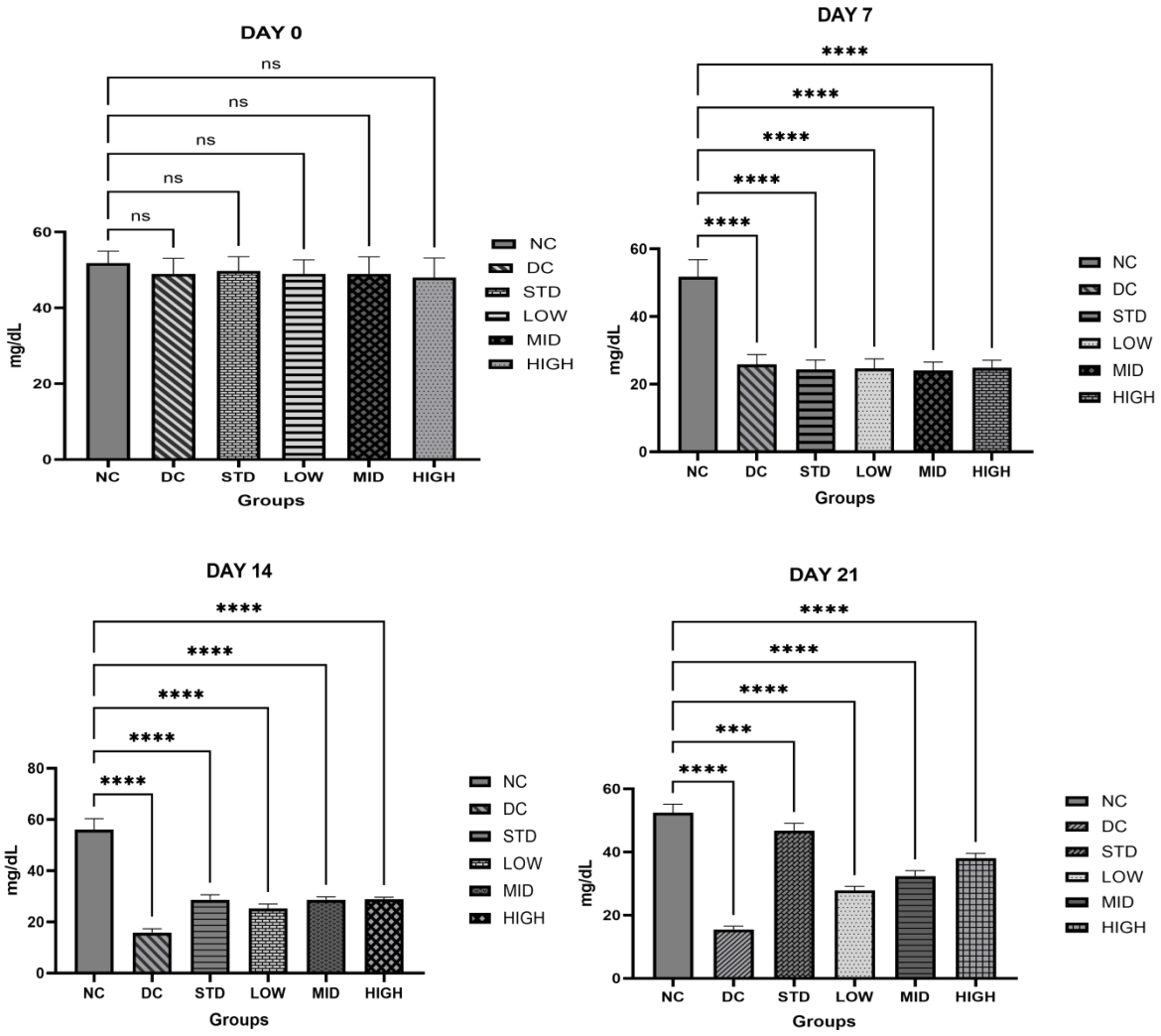


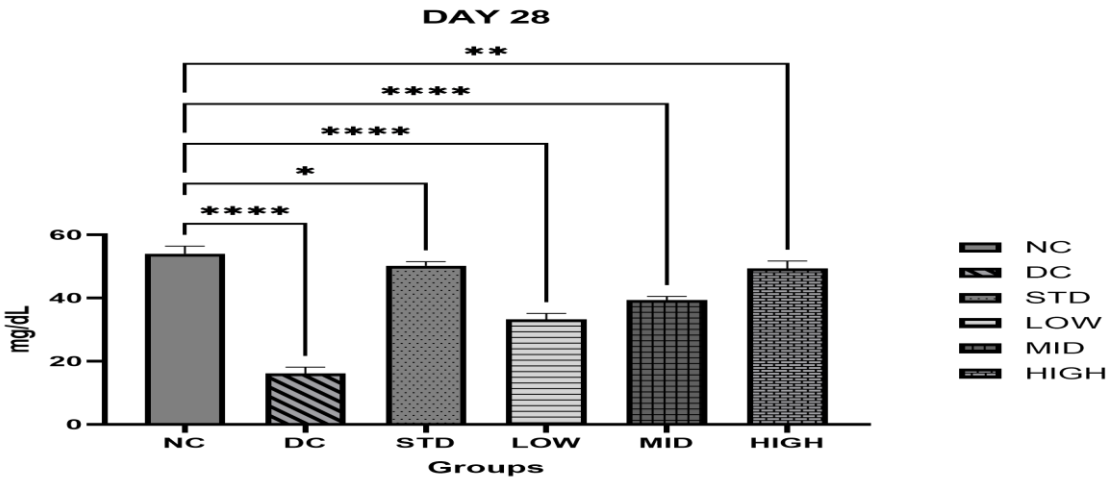






- **Effect of ALE on HDL:**

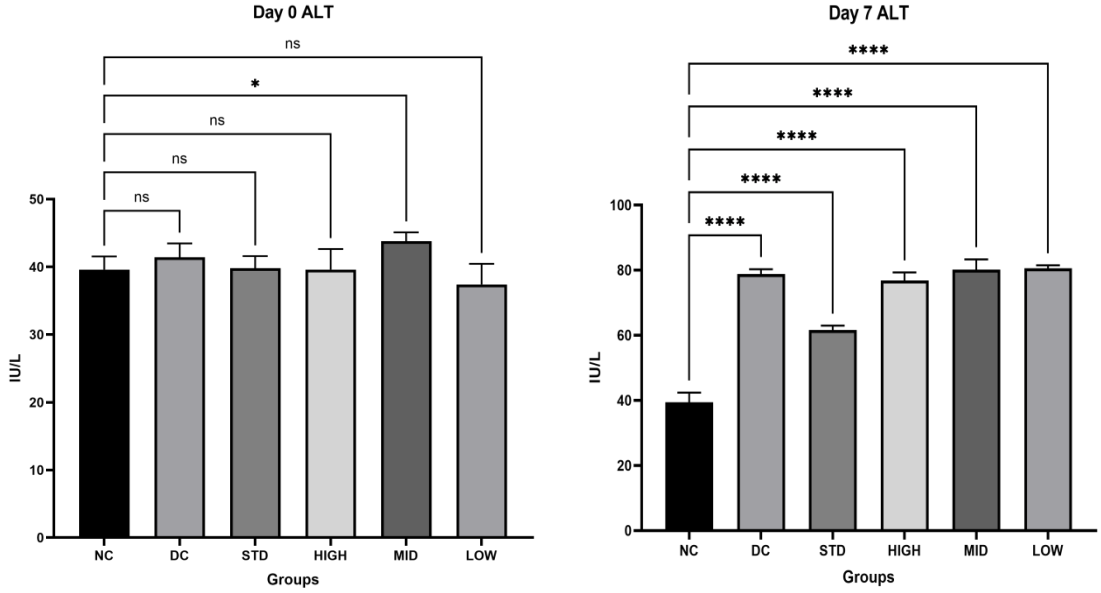


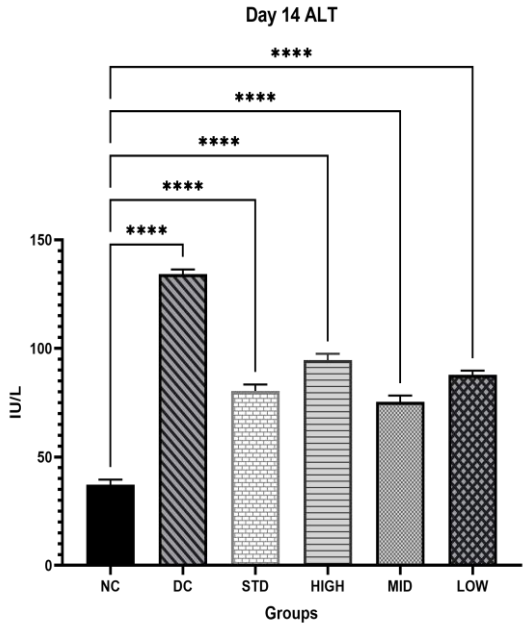


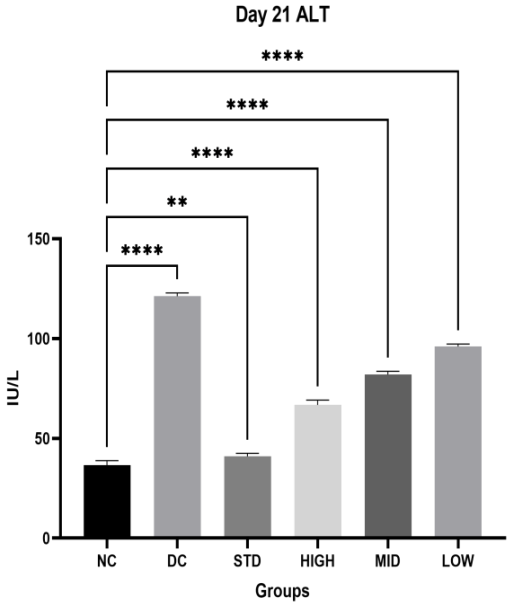
8.8.4Estimating the parameters for the serum liver function test:

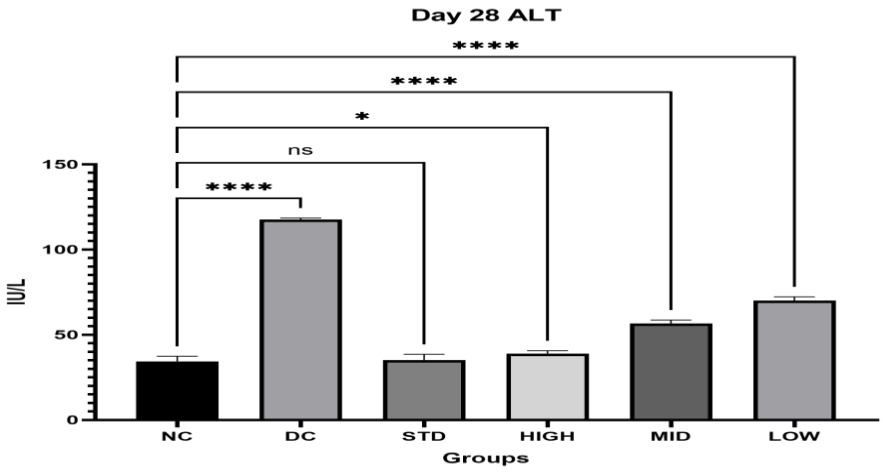
In comparison to the normal control group, the STZ-induced diabetic rats showed considerably ($p < 0.05$) raised biochemical markers such as ALT and AST as well as significantly ($p < 0.05$) reduced total protein content. When compared to the diabetic control group, treatment with ALE at dosages of 100, 200, and 400 mg/kg substantially ($p < 0.05$) decreased the levels of ALT and AST.

- **Effect of ALE on ALT:**

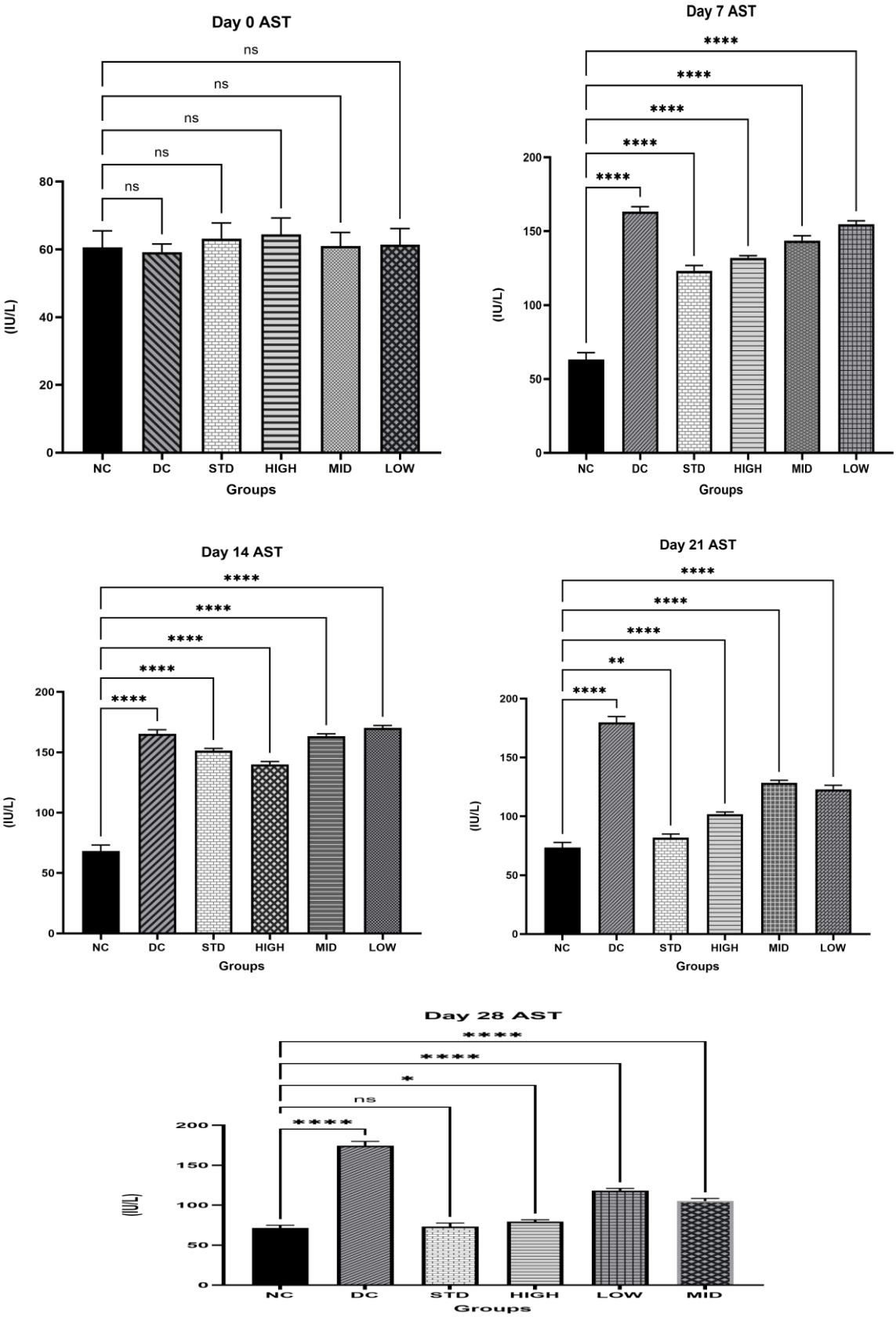



Day 14 ALT
****
****
****
****
****
150
100
50
0
IU/L
NC
DC
STD
HIGH
MID
LOW
Groups


Day 21 ALT
****
****
****
**
****
150
100
50
0
IU/L
NC
DC
STD
HIGH
MID
LOW
Groups


Day 28 ALT
****
****
*
ns
****
150
100
50
0
IU/L
NC
DC
STD
HIGH
MID
LOW
Groups

- **Effect of ALE on AST:**



8.8.5Liver Weight after Sacrifice:

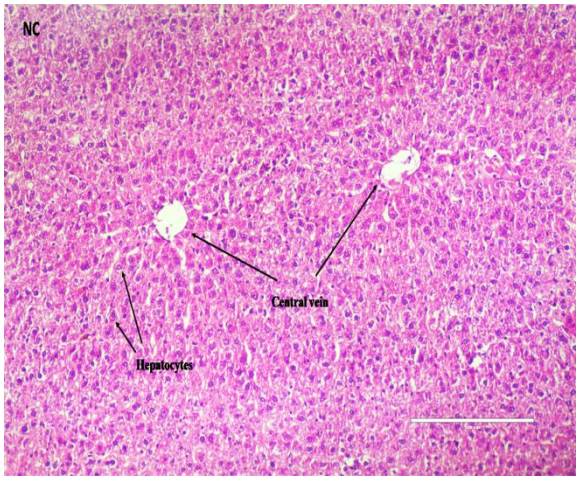
Si.No	Group Name	Liver weight
1.	NC	6.7±2
2.	DC	20.2±3
3.	Low	15.5±2
4.	Medium	12.3±2
5.	High	9.3±3
6.	STD	8.5±2

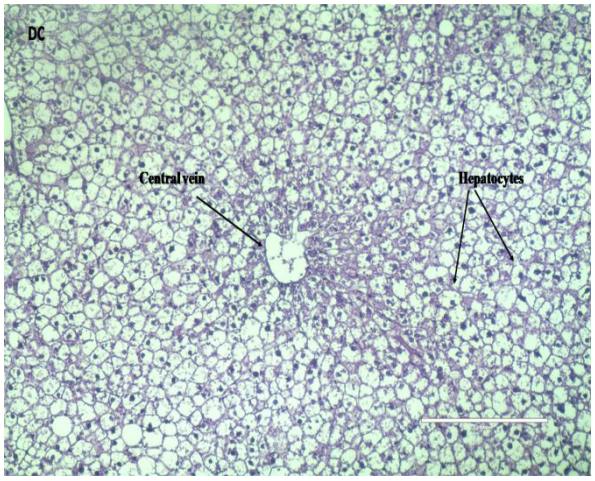
Values are mean ± SD, (n= 3)

8.8.6Histopathological studies:

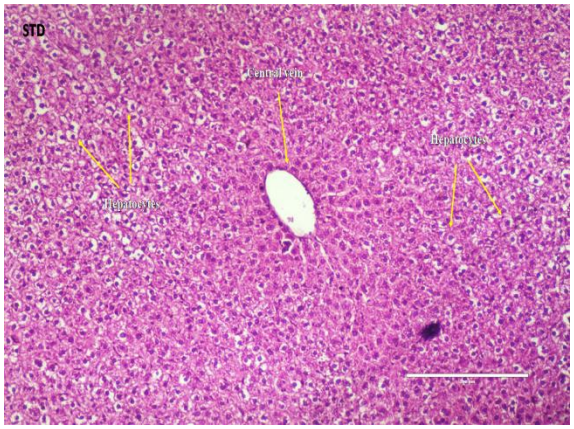
- **H&E Studies:**

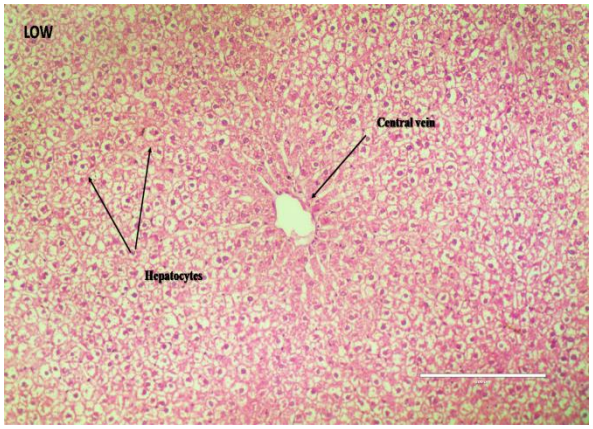
The histological study of the liver in Disease group rats revealed significant deterioration of the liver tissue. Treatment with a High dose of ALE extract (400 mg/kg) enhanced the cellular structure, with visible central veins surrounded by hepatocytes and well-organised hepatic ducts, indicating that the plant extract has hepatoprotective properties.



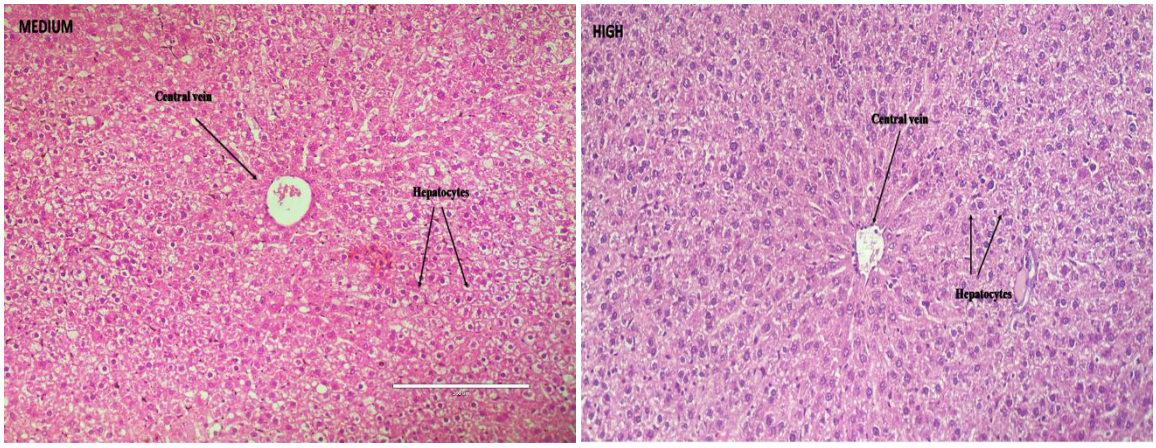


NC **DC**





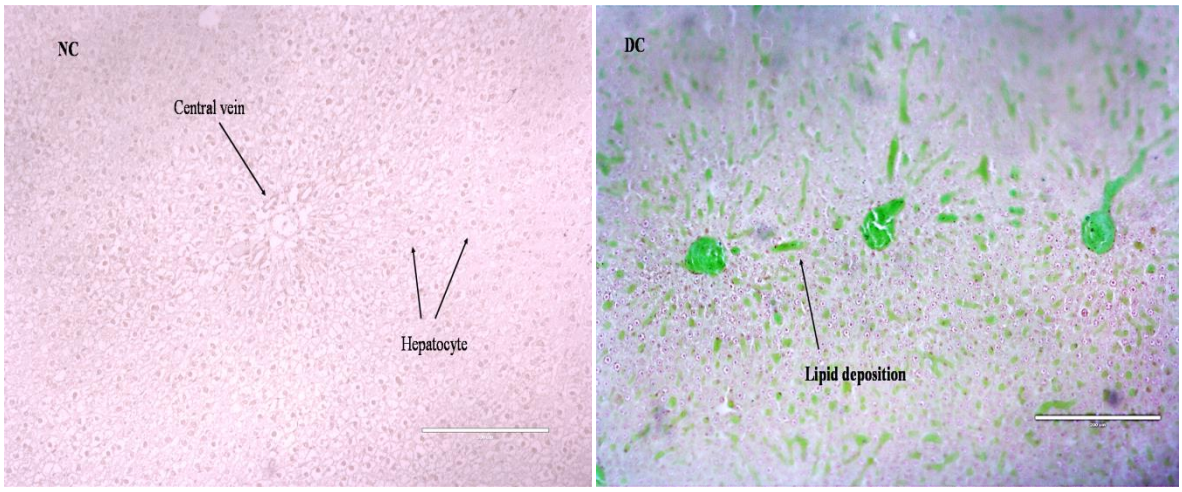
STD **LOW**



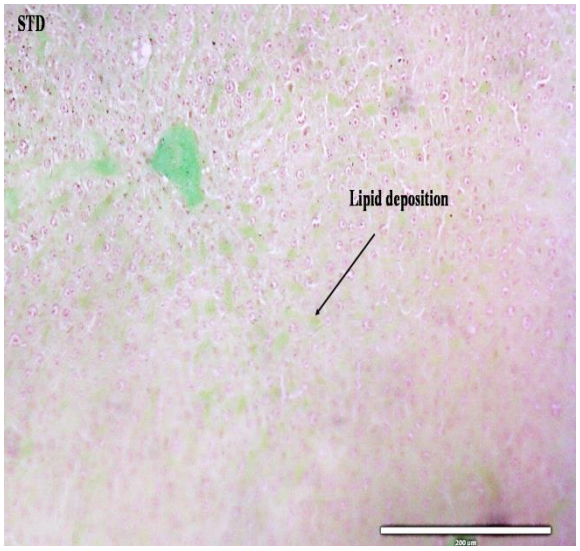
MEDIUM **HIGH**

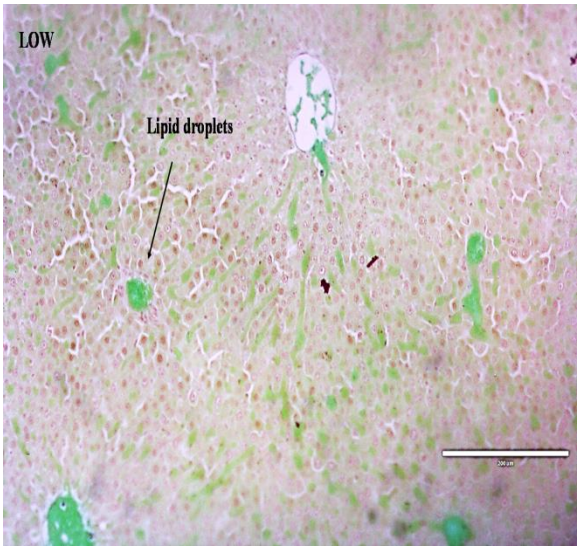
- **Oil red O studies:**

Oil Red O is a fat-soluble dye that stains neutral lipids, cholesteryl esters, and lipoproteins. It has been frequently used for intracellular lipid staining and tissue staining because of its cellular permeability. Neutral fats, primarily triglycerides, can be stained with an orange-red colour. The Oil O Red histological study revealed the significant deposition of fat in the liver tissue of Disease groups. Treatment with a low dose of ALE (100 mg/kg)did not result in a significant reduction in lipid deposition. A medium dose of ALE (200 mg/ kg) shows intermediated lipid levels. However, the treatment with a Standard drug and a High dose of ALE (400 mg/kg) significantly reduced lipid deposition level.

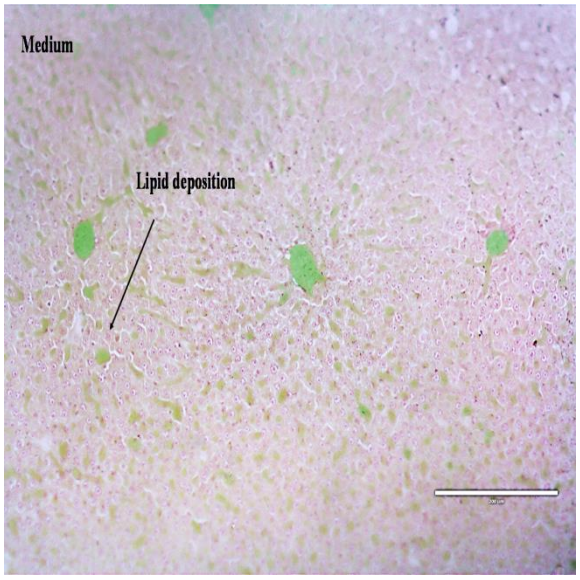


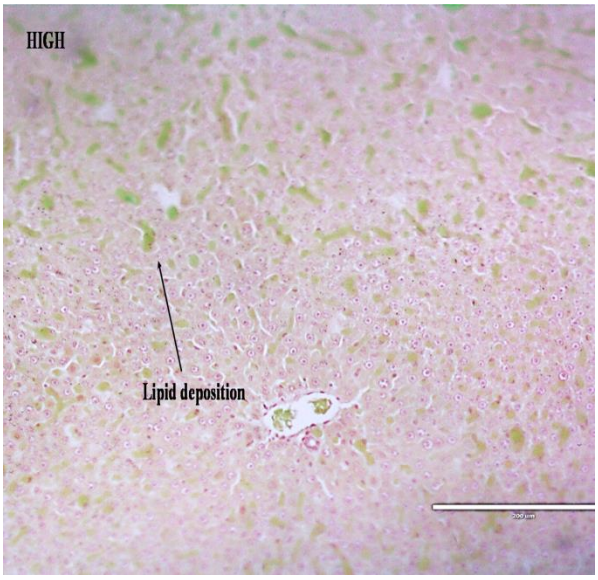
NC **DC**





STD **Low**



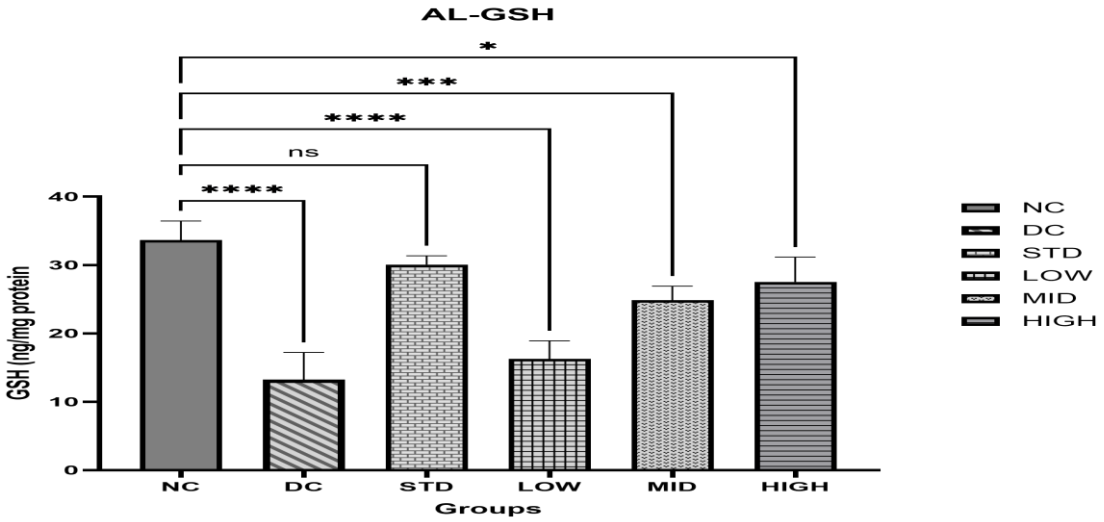


Medium **High**

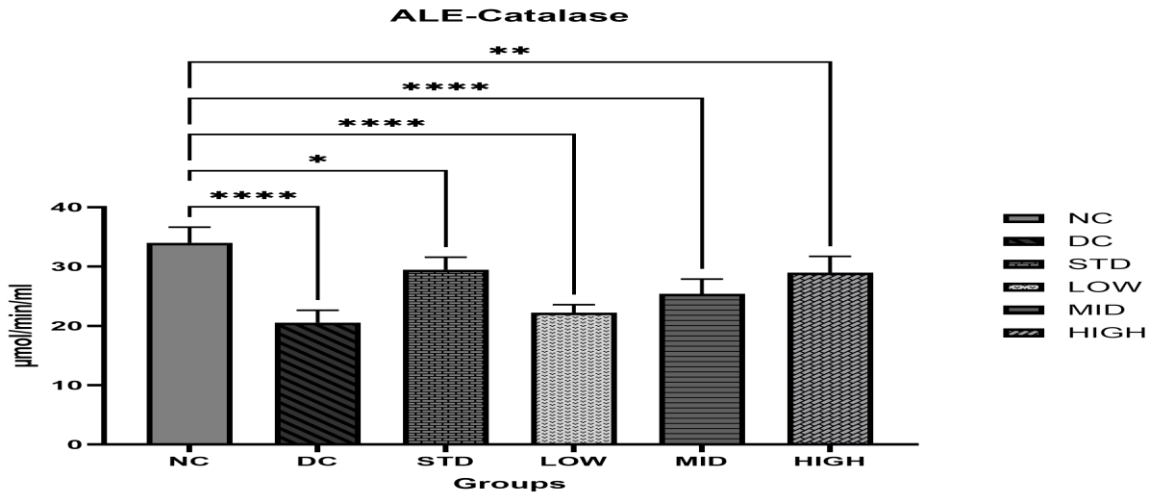
8.8.7Estimation of liver antioxidation level:

The hepatic levels of GSH and catalase activity were significantly ($p < 0.05$) reduced upon induction of T2D, and the MDA level was exacerbated concurrently. After receiving ALE infusion therapy, these levels and activity were markedly restored. Rats given the highest dosage (400 mg/kg bw) showed even greater enhanced activity, on par with those receiving metformin treatment. Hepatic oxidative indicators were either hardly affected or not affected at all when the infusion was given to normal animals.

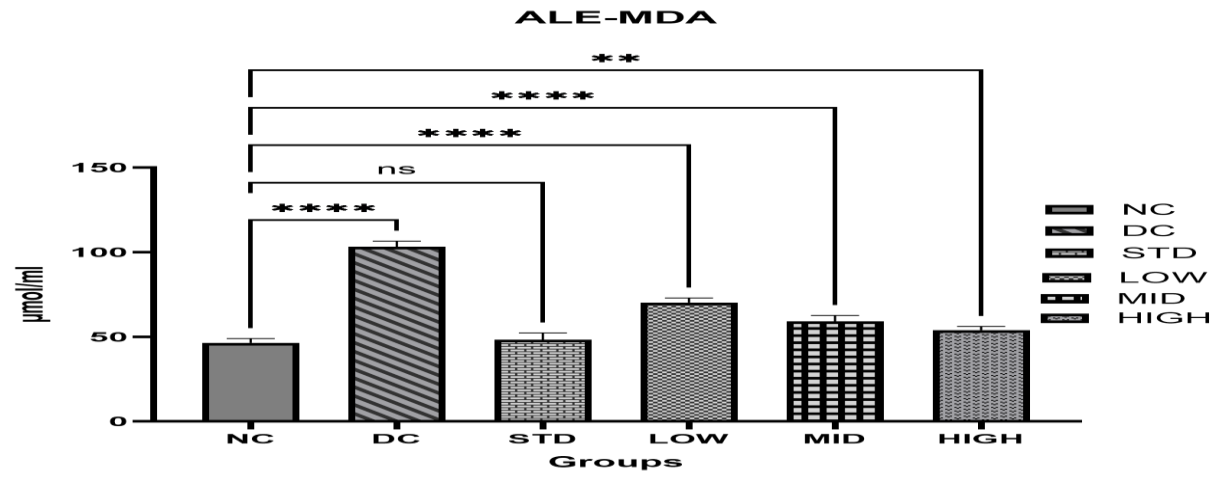
- **Effect of ALE on GSH:**



- **Effect of ALE on catalase:**



- **Effect of ALE on Lipid peroxidation (MDA) levels:**



DISCUSSION

Artocarpus lakoocha, a plant with vital nutritional, medicinal, and economic value, is one of the many plants rich in phytochemicals, particularly oxyresveratrol, which has a range of pharmacological characteristics.The health benefits of flavonoids extend to metabolic illnesses such as diabetes, cancer, obesity, and cardiovascular disease. They also have anti-oxidant properties that mitigate the effects of nitrogen and oxygen species on the body, hence lowering oxidative stress and averting illness. Insulin secretion, glucose absorption, and fat deposition are all regulated by flavonoids' anti-diabetic properties. As a result, there are several polyphenols in A. lakoocha ethyl acetate extract (ALE). Further study is needed to go further into the mechanism of action on NAFLD.

NAFLD is a severe condition that may result in morbidity and death due to micro and macrovascular consequences. Liver dysfunctions, such as liver failure, are the primary cause of death associated with diabetes. The pathophysiology of NAFLD is associated with hyperglycemia caused by insulin resistance and pancreatic β-cell dysfunction. In T2D rats, A. lakoocha infusion has been shown to reduce hyperglycemia, enhance insulin production and utilization, and lessen pancreatic β-cell dysfunction. In this work, A. lakoocha infusion's therapeutic efficacy and mechanism on NAFLD in T2D rats are examined(Mantovani et al., 2022).

Oxidative stress, caused by excess hyperglycemia-mediated free radical production, is linked to the pathogenesis and pathogenesis of nonalcoholic fatty liver disease (NAFLD). In untreated diabetic rats, depleted GSH and catalase activity and increased MDA levels indicate a weakened antioxidant enzyme defence system, leading to increased hepatic oxidative stress in T2D. Antioxidants have been reported to protect against NAFLD by scavenging free radicals and modulating the endogenous antioxidant defence system. Treatment with A.lakoocha infusion improves the endogenous antioxidant defence system, resulting in enhanced hepatic antioxidant activity in diabetic rats(Caturano et al., 2023).

Under normal circumstances, free fatty acids (FFAs) are essential for producing ATP, but under other circumstances, they may convert to alternative substrates like glucose, lactate, and ketone bodies. Chronic hyperglycemia states often lose this flexibility energy switch due to the down-regulation of hepatic GLUT4, leading to increased hepatic lipase activity and lipotoxicity. Increased hepatic lipase activity in untreated diabetic rats is indicative of a raised hepatic FFA level; this, in turn, causes an imbalance between the synthesis of ATP and the use of substrates in diabetic tissues, which is corroborated by increased hepatic ATPase activity. Additionally, diabetic rats receiving an A. lakoocha infusion have reduced lipase activity.

Normalizing ALT and AST levels was necessary since the present study's inclusion of diabetes caused a noticeable increase in the quantity of fat accumulation in the liver. Moreover, DC rats increasing TC, TG, LDL, VLDL, AST, and ALT levels show gradual liver damage.

Pathological examination is critical for detecting NASH, which includes steatosis, lobular inflammation, and hepatocellular ballooning. However, inadequate interobserver agreement might impede assessment. Clear criteria and immunohistochemistry staining for inflammation and ballooning might be helpful.ALE at 400mg/kg demonstrates excellent results by reducing steatosis, lobular inflammation, and hepatocellular ballooning.The study examined changes in rat liver cells and structure after 28 days, confirmed by histopathological (H&E, Oil-O-Red)

investigations. The livers of the normal control and standard groups showed normal hepatocytes with no inflammation or fat deposition. The disease-control group showed hepatocyte deterioration, inflammation, and lipid deposition. A lower dosage of ALE (200 mg/kg) protected the liver, with minor degenerative alterations in hepatocytes. A higher doseof ALE (400 mg/kg) showed liver protection, with a standard hepatocyte shape and no lipid droplet or inflammatory changes.

The present investigation looks at the hepatoprotective, anti-inflammatory, & anti-hyperglycemic effects of Artocarpus lakoocha ethyl acetate extract in diabetic rats. It was shown that the STZ-induced diabetic untreated rats had considerably lower body weight, HDL, liver antioxidants (GSH, catalase) and substantially higher levels of FBG, TG, TC, and MDA when compared to the NC group rats. Treatment with ALE at 400 mg/kg dosages significantly returned serum and liver biochemical markers, body weight, and blood glucose levels to normal. On the other hand, therapeutic dosages of 200 mg/kg were only marginally adjusted, while doses of 100 mg/kg were not significantly normalized(Zhao, 2022).

CONCLUSION

The research assesses the ethyl acetate bark of Artocarpus lakoocha bark's ability to treat NAFLD. The integrative study, including clinical, in vivo, and in vitro research, validates the extract's safety and effectiveness in treating the pathophysiology and development of NAFLD.By efficiently scavenging free radicals and lowering oxidative stress markers, the phytochemical analysis of ALE extract uncovered a rich composition of bioactive compounds, such as polyphenols, flavonoids, and terpenoids, which have significant antioxidant properties in vitro and are essential for reducing oxidative damage caused by NAFLD.Studies conducted in vivo on Wistar rats demonstrated the extract's capacity to alter NAFLD metabolic pathways, lowering fat buildup and boosting anti-inflammatory qualities. Moreover, hepatic steatosis—a sign of NAFLD—was shown to diminish in a dose-dependent manner. According to these results, the extract may be able to treat the inflammatory aspect of the aetiology of NAFLD.In vivo studies on NAFLD animal models showed that ALE extract reduced liver fat content, improved liver function tests, and attenuated histopathological changes. It also improved insulin sensitivity and lipid profiles, which are often deranged in NAFLD patients, demonstrating its beneficial impact on metabolic parameters.In a small-scale in vivo experiment, patients who received ALE extract showed improvements in their quality of life ratings, decreased amounts of hepatic fat, and elevated liver enzyme levels as compared to the placebo group. The extract may be used as a supplemental treatment method in NAFLD therapy, given its safety and tolerability characteristics.The findings suggest a promising extract for treating NAFLD. However, larger-scale, randomized controlled trials are needed to confirm its efficacy and safety, with future studies focusing on optimal dosing regimens, long-term effects, and potential interactions.

Further research is needed to understand the exact mechanisms of action of ALE extract, which has potential pathways like antioxidant, anti-inflammatory, and metabolic modulation effects. A comprehensive understanding of molecular targets and signalling cascades would optimize its therapeutic application. This study contributes to the growing body of evidence supporting plant-based remedies for complex metabolic disorders like NAFLD, emphasizing the importance of exploring traditional medicinal plants as novel therapeutic agents.The ethyl acetate extract of Artocarpus lakoocha bark is promising as a therapeutic agent for managing NAFLD due to its multi-faceted effects on oxidative stress, inflammation, and lipid metabolism. Although further research is needed to establish its clinical efficacy and safety, this study provides a solid foundation for developing a novel plant-based intervention in NAFLD treatment.

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