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Evaluation Of The Antihyperlipidemic And Anti Oxidant Activity Of Albizia Lebbeck Extract In Wistar Rats

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Abstract

Background: *Hyperlipidemia is a chronic metabolic disorder associated with cardiovascular complications including atherosclerosis, ischemic heart disease, and myocardial infarction. Despite the availability of synthetic lipid-lowering agents, their long-term use is limited due to side effects and cost.*

Objective*: The present study investigates the antihyperlipidemic and antioxidant potential of Albizia lebbeck seed extract in experimental Wistar rats.*

Methods*: Albizia lebbeck seeds were extracted using ethyl acetate in a Soxhlet apparatus. Physicochemical and phytochemical evaluations were performed, including TLC and FTIR analyses. Acute and sub-acute toxicity studies were conducted following OECD guidelines. Hyperlipidemia was induced in Wistar rats using a high-fat, high-fructose diet, followed by administration of the extract at different doses (500, 1500, and 2000 mg/kg).*

Results*: Phytochemical screening confirmed the presence of flavonoids, alkaloids, glycosides, and saponins. Toxicity studies revealed no adverse effects on body weight, food/water intake, hematological or biochemical parameters, or organ histopathology. Treatment with A. lebbeck significantly reduced serum cholesterol, triglycerides, LDL, and VLDL levels, while increasing HDL.*

Conclusion*: Albizia lebbeck seed extract demonstrated significant antihyperlipidemic and antioxidant activity without notable toxicity, suggesting its potential as a safe herbal therapeutic.*

Keywords*: Hyperlipidemia, Albizia lebbeck, Herbal extract, Antioxidant, Wistar rats*

INTRODUCTION

Hyperlipidemia is a major metabolic disorder that has emerged as one of the most significant risk factors for cardiovascular diseases worldwide. It is characterized by elevated levels of plasma lipids, including cholesterol, triglycerides, and low-density lipoproteins, which accelerate the development of atherosclerosis. This condition, in turn, predisposes individuals to ischemic heart disease, coronary artery disease, myocardial infarction, and ultimately heart failure. According to global health statistics, cardiovascular diseases linked to hyperlipidemia account for millions of deaths annually, making it a pressing public health concern.

Although several synthetic lipid-lowering agents such as statins, fibrates, bile acid sequestrants, and cholesterol absorption inhibitors are clinically available, their long-term use is often restricted. This is primarily due to side effects including liver dysfunction, muscle toxicity, gastrointestinal disturbances, and the high cost of therapy. Furthermore, not all patients achieve desired lipid control with existing drugs, highlighting the need for novel, safer, and more effective therapeutic strategies.

The three primary lipids found in the body are cholesterol, triglycerides (TGs, also known as triacylglycerides), and phospholipids. Durrington et al [1]. Triglycerides and cholesterol, which are lipids, need to be transported through the bloodstream with the help of lipoproteins, which are proteins, because they are not soluble in water. In order to avoid toxicity, it is necessary to provide higher amounts of fatty acids from meals in the form of triglycerides. Lipoproteins are essential for the uptake and movement of nutrients by the small intestine, as well as for the transfer of lipids from the liver to peripheral tissues and from the gut to the liver. Feingold et al [2]. Lipids control the movement of molecules between the membranes of different organelles, which helps to define their unique properties and functional makeup. As second messengers, they also maintain reserve energy stores in the form of lipid droplets and initiate specific cellular activities Anand et al [3]. Hyperlipidemia is a term used to describe a range of inherited and acquired conditions characterized by elevated levels of lipids (fats) in the body. It is particularly prevalent in the Western hemisphere. Clinically, hyperlipidemia is defined as having levels LDL, TC, TG, or lipoproteins that exceed the 90th percentile compared to the general population, or having levels of HDL that fall below the 10th percentile. Lipids encompass cholesterol, lipoproteins, LDL, and HDL Hill et al [4]. Compared to individuals at low or moderate risk (48%), patients at high or very high cardiovascular risk (72%) more commonly needed pharmacological therapy to address hyperlipidemia Nouh et al [5]. A rise in plasma lipid concentration linked to hyperlipidemia can arise from either acquired or hereditary factors. Hyperlipidemia, in any form, is associated with a greater risk of pancreatitis, early atherosclerosis, and chylomicronemia syndrome. The main cause of the primary form of hyperlipidemia is a genetic impairment in lipid metabolism. A lipid-rich or poor diet, obesity, hypothyroidism, glucose intolerance, smoking, liver disorders, cigarette, alcohol intake, and other factors are major causes of acquired hyperlipidemia (AH).

In recent years, herbal medicines and plant-based formulations have gained recognition for their potential in managing chronic diseases, owing to their wide availability, relatively low toxicity, and multifaceted pharmacological effects. Albizia lebbeck, a leguminous plant widely used in Ayurveda and traditional medicine, has been reported to possess antioxidant, anti-inflammatory, and cardioprotective properties. Despite these documented activities, its role in lipid regulation and prevention of hyperlipidemia has not been fully elucidated.

The present study aims to bridge this gap by preparing an ethyl acetate extract of Albizia lebbeck seeds and evaluating its safety and therapeutic potential in experimental models of hyperlipidemia. Acute and sub-acute toxicity assessments were conducted following OECD guidelines to confirm its safety profile. Subsequently, a high-fat, high-fructose diet model was employed to induce hyperlipidemia in rats, and the effects of the extract on lipid parameters and overall metabolic health were investigated. This study, therefore, provides new insights into the potential application of Albizia lebbeck seed extract as a safe and effective herbal remedy for the management of hyperlipidemia.

Urbanization is linked to an increase in the consumption of foods high in fat and energy, as well as a decrease in physical activity, which might result in AH Rai et al [6]. The ability to assess the lipid content of HDL in numerous individuals and conduct extensive epidemiological research on the relationship between HDL-C concentrations and CAD was made possible by developments in the precipitation of apoB-containing lipoproteins Rader et al [7]. Being hyperlipidemic poses a major health risk. According to studies, hyperlipidemia plays a role in the development of neurodegenerative diseases like AD and PD, as well as a number of other diseases like stroke, atherosclerosis, coronary heart disease, diabetes, myocardial infarction, and pancreatitis Xiang et al [8].

Microvascular function is regulated by a range of lipids, including HDL, LDL, TG, and TC. In addition to causing capillary endothelial cell death and a reduction in coronary blood flow reserve and capillary density, hypercholesterolemia also impairs left ventricular (LV) function. It is suggested that high cholesterol may affect

the lipid bilayer of the membrane, control intracellular calcium ions, and alter the isoform expression patterns of myosin heavy chain, thus increasing the myocardium's susceptibility to external injury Yao et al. [9].
The largest lipoprotein particles, chylomicrons, are produced in the intestinal wall as a reaction to dietary fat. Chylomicrons move cholesterol and dietary triglycerides—which account for over 90% of their mass—out of the colon and into the bloodstream. They include Apolipoproteins (apo) play crucial roles in lipid metabolism. Apo E and the apo Cs are synthesized in the liver and transported to chylomicrons. In contrast, apo B-48, A-I, and A-IV are produced in the intestines. When chylomicrons reach the capillaries of skeletal muscle and adipose tissue, they encounter lipoprotein lipase, an enzyme activated by apo C-II on the surface of chylomicrons. Lipoprotein lipase hydrolyzes the triglycerides in the core of chylomicrons into fatty acids. These fatty acids are then taken up by adipocytes and muscle cells, where they can either be oxidized for energy or re-esterified into triglycerides for storage. This process is essential for the distribution and utilization of dietary fats throughout the body. The remaining chylomicrons, often referred to as remnant particles, are absorbed by the liver in a manner that is somewhat regulated by apo E. They are comparatively richer in cholesteryl esters but poorer in triglycerides. Triglycerides from food are therefore sent to adipose tissue, and cholesterol to the liver Gotto et al. [10]. In clinical practice, hyperlipidemia often involves elevated levels of triglycerides and/or cholesterol alongside reduced levels of HDL. Many patients exhibit high LDL-C, low HDL-C, and elevated triglycerides due to increased synthesis of VLDL in the liver. Factors contributing to this include genetics, high-carbohydrate diets, excessive alcohol intake, insulin resistance, obesity, and nephrotic syndrome. The risk of atherosclerotic cardiovascular disease (ASCVD) is closely associated with LDL levels, influenced by both the absolute amount of LDL cholesterol and the duration it remains elevated. This underscores the importance of managing LDL-C levels as a primary target in reducing cardiovascular risk. Mittal et al [11].

Type 1-Familial Hypercholesterolemia (FH):
The most prevalent autosomal-dominant genetic condition, familial hypercholesterolemia (FH), affects around 30 million people globally Beheshti et al [12]. LDL-C levels that are unusual high are the hallmark of FH, which is conventionally classified as a monogenic form of chronic hypercholesterolemia. Since FH is an inherited form of hypercholesterolemia, most affected persons are born with hypercholesterolemia. As a result, they are revealing high levels of LDL-C for a long period of time, which increases the risk of early atherosclerosis and its effects significantly if treatment is not received. The extra risk linked to this genetic disease may be reduced and frequently eliminated with early identification and appropriate treatment. Genetic variations causing ineffective liver clearance of circulating LDL particles are the cause of familial hypercholesterolemia (FH) Rocha et al [13]. These patients fit our traditional definition of FH and have a plausible reason for their elevated LDL cholesterol level. Some have conventional physical results Khera et al [14].
1.3.2 Type-2 Familial Combined Hyperlipidemia (FCHL):
A prevalent hereditary condition known as familial combination hyperlipidemia (FCHL) is characterized by elevated fasted blood cholesterol, triglycerides, and apolipoprotein B-100. Next-generation sequencing and other molecular genetic approaches have proven to be highly effective in locating uncommon variations with moderate-to-large effects Taghizadeh et al [15]. In lipid clinics, patients with combined hyperlipidemia (CHL) are frequently seen. Although there are many definitions of CHL, the condition is characterized by concurrent elevations in triglyceride (TG) and LDL-C. Patients with CHL are more likely to develop atherosclerotic cardiovascular disease (ASCVD) and are frequently linked to other co-morbidities, such as type 2 diabetes and obesity Gill et al [16]. The most prevalent primary atherogenic dyslipidemia, familial Combined Hyperlipidemia (FCHL), is typified by excess formation of VLDL and variations in the blood lipid profile, making it challenging to measure LDL-C in clinical settings. Three phenotypes—isolated hypertriglyceridemia, mixed dyslipidemia, and isolated hypercholesterolemia—are indicative of familial combined hyperlipidemia (FCHL), with high levels

of apolipoprotein B (Apo B) being one of the main contributing factors Vargas-Vázquez et al [17]. Additional factors contributing to the FCHL phenotype are mutations in the genes encoding for apolipoprotein E (APOE) and the LDLR, which cause familial dysbetalipoproteinemia (FD) and certain hypertriglyceridemic types of familial hypercholesteromia (FH), respectively Solanas et al [18].

1.3.3 Type-3 Familial Hypertriglyceridemia (FHTG):
FHTG is a rare form of initial dyslipidemia that is inherited. FHTG is defined by slightly raised blood triglycerides; it seldom shows symptoms in children and typically occurs without severe hypercholesterolemia Tullu et al [19]. One type of HTG for which the clinical and genetic spectrum is unknown is familial hypertriglyceridemia (FHTG). FHTG is often misdiagnosed as HTG caused by various factors. This includes mutations in the LPL gene or other genes that control the activity of this enzyme, such as APOC2 and LMF1. Mutations in the LPL gene can cause familial chylomicronemia syndrome (FCS). This encompasses ailments like familial combination hyperlipidemia (FCHL), CHTG is characterized by a typical lipid profile that includes elevated levels of triglycerides. Diagnosis of dyslipidemia is indicated by variations in lipid levels, elevated apo B values exceeding the 90th percentile for age and gender in the general population, and a first-degree family history of early cardiovascular disease Cruz-Bautista et al [20]. Hypertriglyceridemia is intimately linked to metabolic syndrome, diabetes mellitus, and obesity. For example, hypertriglyceridemia coexists with type 2 diabetes in as many as 50% of cases. Apart from this, there is often a genetic tendency that results in hypertriglyceridemia when combined with lifestyle variables. This propensity can span a broad range of serum TG levels and is typically polygenic. The range encompasses a disposition that solely results in hypertriglyceridemia when there is significant obesity and/or alcohol consumption, as well as extremely rare and serious mutations (such as lipoprotein lipase and apolipoproteins A5, CII, and CIII) that may cause extremely severe hypertriglyceridemia in children Parhofer et al [21].

Causes of Hyperlipidemia
There are various different causes of development of Hyperlipidemia. Some of them are listed below.
Genetic Hyperlipidemia:
Hyperlipidemia is a metabolic syndrome. It occurs due to the elevated levels of LDL and Triglycerides Ruixing et al [22]. Numerous environmental influences interact with multiple genes to generate complex illnesses. These illnesses are widespread among the populace and are a significant cause of illness in Western countries. Elevated blood triglyceride, total cholesterol, or both are the hallmarks of familial combination hyperlipidemia (FCHL). According to estimates, the prevalence of this condition ranges from 1% to 2% in Western nations. Furthermore, FCHL is present in 14% of people with premature coronary heart disease (CHD), making it one of the most prevalent genetic dyslipidemias that underlie premature CHD. The complicated FCHL phenotype may be influenced by both environmental and genetic variables Pajukanta et al [23].
Obesity:
According to roughly calculation from the WHO, 400 million persons worldwide have a body mass index (BMI) of 30 or above, and 1.6 billion adults out are heavyweight. The National Health and Nutrition Education Survey (NHANES) from the most current year indicates that two thirds of American people are overweight or obese Charlton et al [24]. People who are overweight frequently exhibit hypertriglyceridemia, decreased plasma levels of HDL-C, and increasing levels of lipoproteins that include apolipoprotein (apo)1 B, such as those found in metabolic syndrome. In comparison to BMI, abdominal obesity—which is typically assessed as waist circumference or waist to-hip ratio, or WHR—is more directly linked to intra-abdominal fat content and more accurately predicts the development of CAD in the future Van et al [25].

Improper Diet and Nutrition:
One source of cholesterol, a subtype of lipid, is produced in the liver and the other is obtained through diet Although the liver uses most of the cholesterol for the manufacture of bile acids, including cholic acid, which is necessary to improve the absorption of hydrophobic substances, tiny amounts of cholesterol are also utilized for the synthesis of steroid hormones and cell membranes. After cholecystokinin stimulation, bile acids are released from storage in the gall bladder and into the duodenum and proximal jejunum. The enterohepatic circulation reabsorbs and recycles approximately 95% of the expelled bile acids, with food intake and synthesis making up the difference. Along with an improper diet and insufficient physical activity, hyperlipidemia, or a metabolic issue, is a widespread condition in modern culture and has been known as the serious risk factor for cardiovascular disease (CVD) Nie et al [26].

Alcohol and Smoking:
It has been established that cigarette smoking is a separate, avoidable risk factor for atherosclerosis and CVDs. Although the frequency of smoking has significantly decreased because to public health intervention efforts, CVDs linked to exposure to ETS are far from extinct. In addition to age, ethnicity, income, and education level, other factors linked to weight gain and a higher risk of CVDs include poor diet patterns, smoking, drinking alcohol, engaging in physical inactivity, sleeping irregularly, and poor access to public health services. Stress at work has also been disregarded as a risk factor for CVDs Lin et al [27]. The most effective approaches for reducing triglyceride (TG) levels involve lifestyle modifications such as abstaining from alcohol, reducing intake of rapidly digested carbohydrates, losing weight, and managing blood sugar levels. However, regardless of the success of these lifestyle changes, the decision to lower low-density lipoprotein (LDL) levels should be guided by assessing the individual's cardiovascular disease risk. Parhofer et al [28].

Diabetes:
The diabetes pandemic of the twenty-first century is expected to affect 600 million people worldwide by 2031. Of those, 90–95% will have obesity, type 2 diabetes is thought to be a important factor in these startling statistics. In addition to the added burden of moderate to severe hyperlipidemia associated with type 2 diabetes, diabetic dyslipidemia is characterized by lower levels of HDL-C and high plasma TG. Retinopathy & nephropathy are among the macrovascular and microvascular problems that these patients are significantly more likely to experience Kowluru et al [29]. Lipid and glucose metabolism are interdependent in numerous ways. Diabetic dyslipidemia, which is characterized by higher triglycerides, HDL-C, the primary clinical manifestation indicating this relationship is the predominance of small, dense LDL particles. A disrupted glucose metabolism might have low HDL-C and hypertriglyceridemia as its cause as well as its result. Moreover, it is now widely known that statins raise the risk of new-onset diabetes by a marginal but noteworthy amount Parhofer et al [30, 31].

Complications linked to badly Hyperlipidemia:
Hyperlipidemia that is not well-controlled can cause a various number of issues that may impact various areas of the body. Here are a few typical complications associated with uncontrolled hyperlipidemia:
Atherosclerosis:
The Greek terms "athero," which means gruel or paste, and "sclerosis," which indicates hardness, are the source of the phrase "atherosclerosis." Everybody who reaches a certain age is genetically predisposed to the disease atherosclerosis Gusev et al [32]. Over a number of years, evidence has developed to support the theory that atherosclerosis involves inflammation fueled by lipids. One significant component that keeps the inflammation in the artery wall going is the gathering and retention of lipoproteins high in cholesterol Dib et al [33].

Coronary artery disease:
CAD is a complex condition influenced by both non-modifiable and modifiable factors. Non-modifiable factors include genetics, family history, age, and gender. Modifiable risk factors encompass lipid levels, obesity, smoking, and psychosocial factors. Fast-paced living has boosted the consumption of unhealthy meals and fast food in the Western world, increasing the prevalence of ischemic heart disease Komilovich et al [34]. A measurement LDL-C of ≥190 mg/dL is considered severe hypercholesterolemia and is linked to a higher risk of CAD Berry at al [35].

Myocardial Infraction:
Globally, myocardial infarction (MI) is the leading cause of mortality. Although mortality rates from MI have decreased globally, heart failure (HF) remains highly prevalent. Weakness is a common complication of cardiovascular disorders, particularly in the elderly, leading to physical impairment, fatigue, decreased walking ability, and reduced physical activity. Inflammatory indicators, hypertriglyceridemia, obesity, or a sedentary lifestyle Salari et al [36]. A heart attack is a medical emergency that is also referred to as a heart attack informally. When there is significant restriction or blockage of blood flow to the heart, a heart attack happens. This occurs extremely quickly because the blood clot obstructs the heart's blood supply and the cardiac muscle's contraction is stopped by low oxygen levels. The buildup of fat, cholesterol, and other materials in the heart's arteries is typically what causes blockages Upadhyay et al [37].

Ischemic Stroke:
Stroke is a leading global cause of mortality and the primary contributor to long-term disability in developed countries. Over the past decade, considerable progress has been achieved in diagnosing and treating acute ischemic stroke to mitigate its effects. Early detection and prompt referral to facilities capable of delivering appropriate care are crucial initial steps in managing stroke patients effectively Phipps et al [38]. There are several separate risk factors for IS that have been found. Among these, diabetes mellitus, atrial fibrillation, hypertension, CAD, congestive heart failure, and problems with lipid metabolism are the most common. Decreasing levels of HDL-C and increasing levels of TC and LDL-C are potential risk factors for IS, according to epidemiologic studies Antonios et al [39].

2. Materials and Methods

2.1 Plant Material and Extraction

Seeds of *Albizia lebbeck* were collected locally, powdered, and extracted with ethyl acetate using Soxhlet apparatus. Extracts were concentrated under reduced pressure and dried.

2.2 Phytochemical and Physicochemical Analysis

- TLC and FTIR analyses performed.
- Ash content, moisture, and extractive values determined.
- Standard phytochemical tests for alkaloids, flavonoids, tannins, etc.

2.3 Animals and Toxicity Studies

- Male Wistar rats used.
- Acute toxicity (OECD 425) and Sub-acute toxicity (OECD 407).
- Doses: 500, 1500, and 2000 mg/kg/day orally.

2.4 Hyperlipidemia Induction

- High-fat diet (coconut oil + vanaspati, 3:1 v/v) and 25% fructose solution for 6 weeks.

2.5 Statistical Analysis

- Data expressed as Mean ± SEM.
- ANOVA followed by post-hoc tests; significance at $p < 0.05$.

Herbal Extract was performed through seed which showed the activity in treatment and prevention of Hyperlipidemia or High Cholesterol. Seeds of Albizia lebbeck was obtained from a local vendor of Kanpur Uttar Pradesh. The seeds were collected and was washed several times to extract out the impurities present in it. Then they were allowed to dry completely under the seed and further study was conducted.

Extraction of Herbal Extract *(Albizia lebbeck)*

The herbal extract of *Albizia lebbeck* was extracted through the Soxhlet apparatus. The powder was placed in a filter paper and place into the extractor, The successive solvent used for extraction was ethyl acetate and the temperature was set to 60-65°C. The whole extraction runs for 4 cycles of the obtained extract were used for further analysis. The Powder of *Albizia lebbeck* 400-500 grams each kept in thimble and extracted with 500-600 ml of ethyl acetate in a Soxhlet extractor in successive manner. Extraction process was continued until the colour of the final drop of the extract become colourless. This extract was concentrated ¼ of the original volume in Vaccum at 60°C using a rotatory evaporator.

Spectral Analysis

4.3.1 TLC Analysis:

Thin layer Chromatography will be performed for the identification of phytochemical constituents. TLC study was carried out on silica gel G. For TLC of *Albizia lebbeck*, extracts are loaded by using mobile phase (water, ethyl acetate, methanol 1:15:1.25)

Name of Compound	Adsorbent	Solvent system	Viewing medium	Rf. Values
Albizia lebbeck	Silica gel G	ethyl acetate: methanol: water, 15:1.25:1	Iodine vapour	0.5

Table 1: Rf Value determination for Thin Layer Chromatography

Fourier Transform Infrared Spectrophotometer (FTIR):

FTIR analysis will be conducted to elucidate the specific functional groups present in the extract. The collected substance was introduced into a Perkin Elmer Spectrum 65 FTIR spectroscope, which possesses a scanning range spanning from 650 to 4000 cm-1.

Physicochemical Evaluation:

The evaluation of different extractive values of herbal extract includes ash content, alcohol soluble, water-soluble ash, acid insoluble ash content was carried out according to the given methods Thomas et al (40).

Total Ash Contant

1–4 g of each *Albizia lebbeck* seed powder into the tared crucibles; 2) placing the crucible safely with sample in the muffle furnace and set it to temperature at 600 *C; 3) leaving the small hole of furnace slightly open to let smoke escape, and closing the door when no more smoke or flame appeared; 4) Scrapping the crucibles with ashed samples from the muffle furnace for cooling in a desiccator; 6) weighing crucible with ash; and 7) calculated the total ash content as % . Liu et al [41].

WI = Empty crucible weight

W2 = Weight of HE including crucible weight for ignition

W3 = Final weight of HE including crucible weight after ignition Total ash content was calculated as:

Total ash (%) = [(W3-W1)/(W2-W1)] *100

Fig 1 Crucible not fully ignite and not became colorless

Fig 2Crucible fully ignite and becomes colorless

Acid Insoluble Ash

The crucible, which held all of the ash, was filled with 25 millilitres of diluted hydrochloric acid (HCl), and subsequently transferred to a beaker. Subsequently, the concoction was heated gradually for a duration of five minutes in a beaker that was sealed with a watch glass. A total of 5 millilitres of water at its boiling point was utilised to cleanse the watch glass, and subsequently, the resulting liquid was transferred into the beaker. In order to produce a neutral filtrate, the insoluble substance was passed through ashless filter paper and subsequently watered with boiling water. Subsequently, the insoluble substance was transferred from the filter paper back to the initial crucible and subjected to drying on a heated surface until the weight (W4) reached a consistent value. Following the process of cooling in a desiccator, the remaining substance was reweighed.

W1 = Empty crucible weight

W2 = Sample weight+ crucible weight for ignition

W3 =Final weight of sample including crucible weight after ignition W4 = Constant weight after addition of HCl

Acid-insoluble ash content was calculated as:

Acid-insoluble ash (%) [(W4-W1)/(W1)] * 100

Moisture Content:
The moisture content was assessed, and the drying loss was evaluated. A porcelain dish, previously prepared and weighed, was filled with 2g (W) of HE. The dish was then placed in a hot air oven and dried until its weight stopped changing or until there was a maximum variation of 0.5 mg between two consecutive weighings. The dish was dehydrated, measured, and subsequently cooled in a desiccator prior to being reweighed.
The percentage of moisture content was evaluated by following equation:

Loss on drying (%) = [(WI-W2)/W] *100 where,

W = Weight of Herbal extract (2g)

W1 = HE (g) before drying

W2 = HE (g) after drying

Identification for Alkaloids
Mayer's test: By the sidewalls of the test tube, two drops of Mayer's reagent were poured to 3ml filtrate. It was discovered that a white creamy precipitate had formed. It was an indication that alkaloids were present.
Hager's test: 1ml of Hager's reagent (Picric acid saturated) mixed to 3ml of filtrate. There was a visible yellow ppt. It was a sign of the existence of alkaloid
Dragendroff's Test: About 0.2 g of 2% H2S04 extract is warned for 2 min. It was filtered and a few drops poured of the Dragendroff's. Orange Red ppt shows the presence of alkaloids.
Identification for Glycosides
Anthraquinone glycosides (Bontrager's test): Sulfuric acid was mixed with extract. After then, the solution was filtered and treated with same amount of chloroform. After that, an equal volume of ammonia solution was introduced. The lack of anthraquinones was shown by the absence of strong pink, violet, or red coloration in the top layer.
Preparation of animals and doses:
The acute oral toxicity assay was screened in Male Wistar rats as per OECD Test Guideline 425. 24 Wistar rats weighing 125-200 grams were divided into 4 groups, each group containing 6 animals. Before the acute screening, all the animals were weighed, marked for identification and fasted overnight (8-12 hours) with free access to water intake. Male Wistar rats in the normal control group received water whereas the dose 1, dose 2 and dose 3 groups received daily doses of 250, 500 and 1000 milli/kilogram body weight per day.
Following the dosing, all the animals were observed keenly for about first 30 min, 4 hours, 24 hours, 7th day and 14th Day. Their weight measurement was taken every week.

Procedure:
Before to dosing, rats were fasted overnight (8-12 hours) but were given water freely. The dose was calculated for each animal and dissolved in the distilled water in desired volume. The animals received their calculated dose daily at the same time by an oral gavage.
Individual food, water consumption, and body weight by rats: Individual body weights (g) were recorded and the consumed amount of water and food were measured, on weekly basis which were recorded as daily food (gm/rat/week) and water consumption (ml/rat/week).
After Completion of Sub-acute toxicity studies, Record of food and water intake, body weights were also done weekly throughout the experimental period. After this on the 29th day of the research study, following an overnight fast of 8-12 hours, Animals were anaesthetized under ether as listed in CCSEA annexure, and blood

samples were collected and then sacrificed by cervical dislocation. Vital organs are isolated like stomach, spleen, liver, brain, intestine and pancreas were stored for histological analysis. The MWR are seen on different parameters which are assessed according to the standard procedures.

HFD leads to the increase in lipid levels, LDL, Triglycerides, VLDL, which increases the chances of getting hyperlipidemia when consumed in a large quantity. SF acid and TFA are commonly known to induce hyperlipidemia. Vanaspati oil contains high level of fatty acids. It contains more than 20% of trans fat and more than 60% of SF. On the other hand, coconut oil contains high proportions of saturated fat. HF consumption increases the likelihood of weight gain and reduces hypertriglyceridemia.
A high fat was created by combining coconut oil and Indian vanaspati ghee in a 3:1 (v/v) ratio. The rats were administered a daily dose of 3 millilitres per kilogramme of body weight. Rats that were consuming a diet high in sugar were administered fructose orally at a concentration of 25%.

RESULTS
Model Design:
The hyperlipidemia model was induced using a diet that is high in fat and sugar (HFHS). Over a period of six weeks, rats were administered a meal consisting of 25% fructose and three millilitres of fat emulsion orally on a daily basis for a duration of twenty-four hours. Once the animals were weighed, recorded, and assigned numerical identifiers, they were distributed into 5 groups, with each group consisting of 6 animals.
This chapter focuses on the analysis and interpretation of data gathered in order to evaluate the efficacy of ALE in the treatment of Hyperlipidemia.
Physicochemical Analysis of ALE

Sr No.	Property	*Albizia lebbeck* (% w/w)
1.	***Ash value***	
	a. Total ash	4.73±2.12
	b. Water soluble ash	1.33±0.005
	c. Acid soluble ash	1.44±0.015
2.	***Moisture content/Loss on drying***	6.19±0.04
3.	***Extractive Value***	
	a. Water Soluble Extractive Value	25.57 ± 0.60
	b. Alcohol Soluble Extractive Value	12.23 ± 0.19

Physico-Chemicals Parameters results of the Herbal extract (HE)

Values are expressed in Mean ± SD (n=6)

High ash values indicate the adulteration, contamination, substitution or carelessness in preparing the drug, The total ash value found to be 4.73± 2.12, which is quite less, it indicates that the obtained ash value is significant and do not contain high traces of silicates, carbonates and phosphates in it. The water-soluble ash and Acid soluble ash should be or less than in 2.0-2.5% range that is approximately same as that of *Albizia lebbeck*. For the seed drug, it is mentioned that Moisture content should be less than 15% and the prepared extract found to be in range of 6.19± 0.04, which is highly significant. The less foreign matter content denotes highest purity in the sample hence the foreign matter content matter in the prepared herbal extract is found to quite less which denotes high purity of the sample. The extractive value is highly significant in the different extracts.

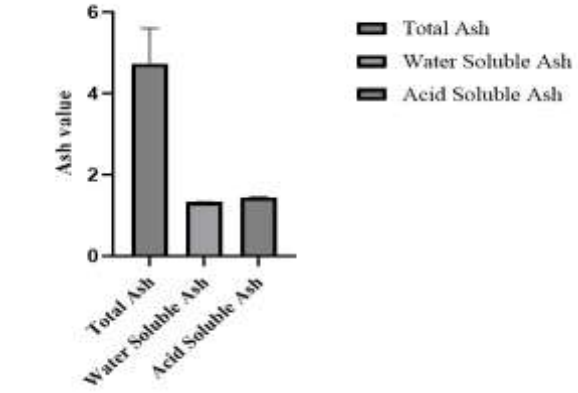
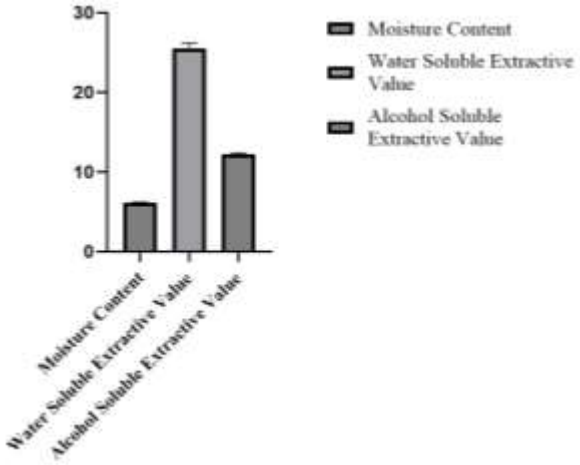


Fig : Ash value



Phytochemical Screening of the ALE

Using qualitative identification tests, it was possible to identify alkaloids, glycosides, flavonoids, tannins, protein, amino acids, steroids and carbohydrates in the Herbal extract as given in Table

Phytochemical Investigation of ALE

Phytochemical Constituents	**Test Name**	***Albizia lebbeck***
Detection of Alkaloids	Mayer's Test Hager's Test Dragendroff's Test	(+) (+) (+)
Detection of glycosides	Anthraquinone glycosides (Bontrager's Test) Saponin Glucosides (Foam Test)	(+) (+)
Detection of Flavonoids	Sulphuric Acid Test	(+)
Detection of Tannins	Nitric acid Test	(+)
Detection of Protein	Millon's Test	(+)
Detection of Amino acids	Ninhydrin Test	(+)
Detection of Steroids	Salkowski Test	(+)
Detection of Carbohydrates	Molish's Test Fehling's Test Benedict's Test	(+) (+) (+)

The Prepared Herbal extract contain a rich source of active phytoconstituents which includes alkaloids, Glycosides, Flavonoids, Tannins, Protein, Amino acid, Steroids and carbohydrates which are identified by the phytochemicals test are mentioned above. The presence of these phytoconstituents result in producing the pharmacological activity for the treatment of Hyperlipidemia.

Effect on Body-Weight Parameters

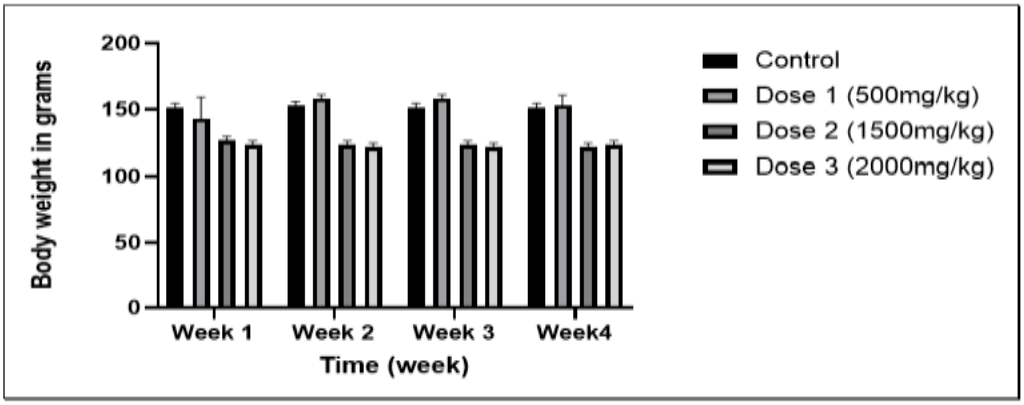
The Body-weight parameters measurements in the given Table 5.5 and represented in Figure 5.5, which did not reveal a significant change in weight of Wistar rats when treated with HE compared to the control group. Highest dose of extract (2000mg/kg) does not show visible change in the body weight of the Wistar rats. In present study all extracts of HE of dose 500 mg/kg, 1500 mg/kg and 2000 mg/kg do not show increase in body weight significantly ($p < 0.0001$) which was evaluated to the body weight of the normal controls. Greater dose of ALE does not show any recognizable signs of toxicity and death after once daily administration orally for 28 days. So, the ALE is safe for longer use.

	Mean ± SEM

Oral treatment (mg/kg)	1st week	2nd week	3rd week	4th week
Control, Saline	10.25±0.5	11.66±2.88	11±1.15	10.5±1.15
500mg/kg	12.5±2.88	9.33±1.15	10.66±1.15	11.33±1.15
1500mg/kg	13.6±0.5	14.33±0.5	14.0±1.73	13.67±1.52
2000mg/kg	14.6±0.5	13.33±2.88	13.33±1.15	12.66±1.15

Group	1st week	2nd week	3rd week	4th week
Control	151.6 ± 2.88	153.3 ±2.88	151.6 ±2.88	151.6 ±2.88
500mg/kg	143.3 ±16.07	158.3 ±2.88	158.3 ±2.98	153.3 ±2.63
1500 mg/kg	126.6 ±2.88	128.3 ±2.88	123.3 ±2.98	121.6 ±2.88
2000 mg/kg	123.3 ±2.88	121.6 ±2.88	121.6 ±2.88	123.3 ±2.88

Table Effect of ALE on body weight changes of MWR in SAOT studies
Values are expressed in Mean ± SD (n=6)
Effect of Herbal Extract (HE) on body weight differs of MWR in SAOT studies during 4 weeks of treatment with HE to different treatment groups and normal water given to control group. Representative figure shows that there is no increase in the BW when compared to control group (***p<0.0001).



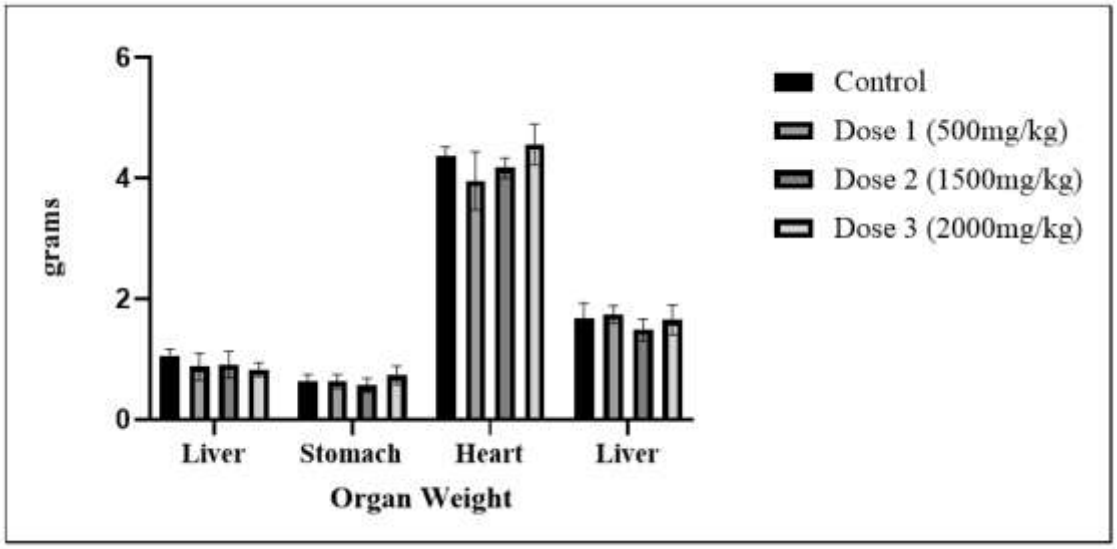
Effect of ALE In MWR on Food and Water Intake
The Food Intake measurements are given in Table 5.6(a) and represented in *Figure 5.6(a)*, which did not reveal a change in food intake of Wistar rats when treated with HE and compared to the control group. Highest dose of extract (2000mg/kg) did not show significant change in the food intake of the Wistar rats. In this study, all extracts of ALE of dose 500 mg/kg, 1500 mg/kg and 2000 mg/kg do not show any noticeable increase in food intake of Wistar rats significantly ($p < 0.0001$) which was comparable to the food intake of the normal controls. Values are statistically significant at $p<0.0001$.

Organ Weight Assessment in Repeated Dose Toxicity Studies in MWR

After 28 Days of study, the animals are sacrificed and organs such as Heart, Liver, Kidney, Stomach are isolated and weighed individually as compared with control group as shown below in Table 5.9. There is no significant change seen in organ weight of the male Wistar rats of different doses 500, 1500 and 2000 milligram/kilogram as compared to that of normal control group.

Organs	Mean ± SEM			
	Control	Dose 1 500 mg/kg	Dose 2 1500 mg/kg	Dose 3 2000 mg/kg
Liver	1.04 ± 0.12	0.87 ± 0.22	0.91 ± 0.21	0.83 ± 0.11
Stomach	0.63 ± 0.11	0.63 ± 0.11	0.56 ± 0.11	0.73 ± 0.15
Heart	4.36 ± 0.15	3.96 ± 0.48	4.17 ± 0.16	4.56 ± 0.33
Liver	1.67 ± 0.26	0.74 ± 0.14	1.48 ± 0.18	1.65 ± 0.25

Organ Weight Assessment in Repeated Dose Toxicity Studies in Male Wistar Rats (Values expressed as Mean ± SEM).



Organ Weight Assessment in Repeated Dose Toxicity Studies in Male Wistar Rats (Values expressed as Mean ± SEM).

Albizia lebbeck seed has significant property in lipid lower when extracted in ethyl acetate. Albizia lebbeck also shows anti-oxidant properties. Ethyl acetate extraction of albizia lebbeck shows presence of flavonoids and saponins which are beneficial in lowing lipid levels Azam et al [42]. Seed of albizia lebbeck contains linoleic acids which is an important unsaturated acid. Presence of linoleic acid in seed prevents cardiovascular disease as it contains polyunsaturated fatty acids which helps in the reduction of lipids and serum cholesterol Ali et al [43].

Extraction of Albizia lebbeck seed was carried out by washing seeds until no impurities and water turns out clear was done. Once the seeds are washed, they were crushed and a fine powder was obtained. Grinded powder was then incorporated in Soxhlet apparatus at 60-65^{0}C with the solvent used ethyl acetate having

increasing polarity. After 3-4 cycles in Soxhlet product in round bottom flask was taken out and fixed in rotatory evaporator to evaporate all the solvent and extracted product was obtained Amog et al [44]. Organoleptic properties of albizia lebbeck seed were brownish in colour had a sticky texture with bitter in taste and have smells of ethyl acetate which was used as a solvent in extraction Fakoya et al [45]

CONCLUSION

As per our study investigation and examination, the results provided us with the conclusion that there is a significant effect of the prepared Herbal extract in treatment of Hyperlipidemia in High Fat- High Fructose induced Hyperlipidemia in experimental Wistar rats. Results are significant in providing the positive effect of the specific herb as stated in the study. It included herb provide the action of alkaloids, flavonoids, Saponins. There were no reported acute or sub-acute toxicity associated with the herbal extract. During the study there was no organ toxicity or significant alteration in hematological or biochemical parameters. Further studies need to be done for the investigation of similar effects in different extracts and different doses of the Albizia lebbeck seed extract as it holds the potential and promising role in the treatment of Hyperlipidemia.

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The % yield of ethanolic extract was calculated using the formula:

% Yield = Weight of extract (g)/Weight of dry powder(g) x100