Investigate Antihyperlipidemic Activity of Crataeva Nurvala Bark by High Fat High Sugar Diet Induced Model

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Abstract—**In order to create a rat model that is appropriate for pharmacological screening, the current study set out to reproduce the natural history and metabolic features of type 2 diabetes in humans. The male Sprague-Dawley rats (160-180 g) were split into two groups and fed either the in-house made high-fat diet (HFD) (58% calories as fat) or the commercially available normal pellet diet (NPD) (12 % calories as fat) for two weeks. Insulin (PI), triglycerides (PTG), body weight, total cholesterol (PTC), and basal plasma glucose (PGL) were significantly increased in the HFD-fed rats when compared to the NPD-fed control rats. The HFD rats also shown a significant decrease in the intravenous insulin glucose tolerance test (IVIGTT) glucose disappearance rate (K-value). Hyperinsulinemia and a decreased glucose disappearance rate (K-value) indicated that rats fed a high-fat diet developed insulin resistance. A subgroup of the rats from both groups received an intraperitoneal injection of a low dose of streptozotocin (STZ) (35 mg kg(-1)) following two weeks of dietary manipulation. When STZ was injected into insulin-resistant HFD-fed rats, they experienced frank hyperglycemia; in contrast, NPD-fed rats experienced a moderate increase in PGL. After receiving a STZ injection, the PI level in HFD rats significantly decreased, but only to a level that was similar to that of control rats fed NPD. Furthermore, following STZ therapy in HFD-fed rats, the levels of PTG and PTC were further exacerbated. However, in rats given NPD, STZ (35 mg kg (-1), i.p.) did not significantly change the levels of PI, PTG, and PTC. These fat-fed/STZ-treated rats thereby mimic the metabolic traits and natural course of the disease that are typical of people who are more likely to acquire type 2 diabetes due to obesity and insulin resistance. Furthermore, it was discovered that both insulinotropic (glipizide) and insulin sensitizing (pioglitazone) medications had the ability to reduce glucose levels in the fat-fed/STZ-treated rats. These diabetic rats also demonstrated the impact of glipizide and pioglitazone on the plasma lipid parameters (PTG and PTC). The current study shows that a combination of a rat fed a high-fat diet and receiving a low dose of STZ can be used as an alternative animal model for type 2 diabetes that mimics the human condition and can be used to test anti-diabetic medications for sort 2 diabetes treatment.**

1. INTRODUCTION

1.1 Hyperlipidemic: The word "hyperlipidemia" refers to a group of inherited and acquired conditions that are characterized by high amounts of lipids in the body. It is a relatively prevalent illness worldwide, but particularly in the Western Hemisphere. Although hyperlipidemia by itself usually does not cause life-threatening symptoms, the presence of this underlying pathology frequently results in severe illnesses that can be fatal. to reduce the incidence of morbidity and death linked to this illness.

An excess of lipids (fats) in the blood is referred to as hyperlipidemia, dyslipidemia, or excessive cholesterol. To aid in food digestion and the production of hormones, your liver produces cholesterol. However, meals from the meat and dairy sections also contain cholesterol. The cholesterol in your diet is excess since your liver can produce as much cholesterol as you require.

The elevated levels of serum TC, VLDL, TGLDL, and HDL are referred to as hyperlipdemia. Heart disease is caused by hyperlipidemia, a disorder of lipid metabolism brought on by an increase in the plasma concentration of various lipid and lipoprotein components. can cause a variety of problems, including myocardial infarction, atherosclerosis, heart attacks, coronary artery syndrome, strokes, and pancreatitis. Lipoproteins are "macromolecular complexes in the blood that transport lipids" because

lipids are insoluble in aqueous conditions and bind to protein moiety as a carrier. Apolipoproteins, which are proteins found on the surface of lipoproteins, are involved in controlling the metabolism and cellular uptake of lipoproteins.

1.2 SYMPTOMS OF HYPERLIPEDEMIC:

The majority of people with high cholesterol don't experience any symptoms. Xanthomas, which are waxy, fatty plaques on the skin, or corneal arcus, which are cholesterol rings surrounding the iris of the eye, can develop in people with a genetic issue with cholesterol clearance that results in extremely high cholesterol levels.

1.3 FACTORS AFFECTING HYPERLIPEDEMIC:

A number of factors can increase your risk of hyperlipidaemia, such as:

A poor diet, such as consuming more than 40% of calories from fat, more than 10% from saturated fat, and more than 300 mg of cholesterol daily, is the primary risk factor for hyperlipidaemia. Other factors that contribute to the condition include changes in lifestyle habits. having a history of elevated cholesterol in the family. Becoming hypothyroid. Being obese. Not consuming a healthy diet. Excessive alcohol consumption. Having diabetes, smoking, excessive sitting as opposed to exercise. Under hardship.

1.4 HOW HYPERLIPEDEMIC AFFECT THE BODY:

If left untreated, hyperlipidaemia (higher cholesterol) can lead to atherosclerosis, or the buildup of plaque inside your body's blood arteries. Complications from hyperlipidaemia may result from this, including: heart attack. stroke. coronary heart disease. Disease of the carotid arteries. Unexpected cardiac arrest. Disease of the peripheral arteries microvascular illness. Promycelia multiplex.

1.5 ANTIHYPERLIPEDEMIC

Antihyperlipidemic medications encourage the blood's lipid levels to drop. Certain antihyperlipidemic medications work to decrease triglyceride levels, some to decrease low-density lipoprotein (LDL) cholesterol, and some to increase high-density HDL cholesterol, or lipoprotein. They can avoid coronary heart disease's primary and secondary symptoms by lowering LDL cholesterol.

1.6 TREATMENT OF HYPERLIPEDEMIC

There are two types of hyperlipidemia: primary and secondary. The first can be managed with hypolipidemic medications, while the latter is more mildly handled by the original condition and is brought on by diabetes, hypothyroidism, or renal lipid nephrosis. Allopathic hypolipidemic medications, such as statins, are available on the market, but they have numerous adverse effects, including hyperuricemia, diarrhea, myositis, hepatotoxicity, and more. Genetic abnormalities and lifestyle choices high in calories, fat, and cholesterol are major contributors to dyslipidaemia worldwide.

They may block other important enzymes in the body because they are primarily enzyme inhibitors. Furthermore, because statins are taken over an extended period of time, they have long-term harmful effects. Therefore, research into spontaneous hypolipidemia is currently receiving a lot of interest. Some people may only need to alter their lives in order to see an improvement in their cholesterol levels. For others, that is insufficient, and they require medicine. Additional everyday pursuits that may be incorporated: Frequent Physical Activity Reducing the frequency of smoking obtaining a minimum of seven hours of sleep every night. Controlling your level of stress. Consuming more nutritious food. Limiting your alcohol intake. Reaching a healthy weight by dropping a few pounds.

2. LITERATURE REVIEW

2.1 Literature related to plant:

2.1.1 Velliyur K Gopalakrishnan et. al. (2012): Antioxidant properties of Crataeva nurvala bark contains a variety of the bioactive phytochemical constituents in medicinal plants which include flavonoids, phenolic compounds, tannins, anthracene derivatives, and essential oils. Components from Crataeva nurvala bark have been accounted to play an important role in scavenging free radicals generated by mutagens and carcinogens. Androgens are the key factors in either the initiation or progression of prostate cancer by inducing oxidative stress. In the

present set of investigations, the antioxidative potential of Crataeva nurvala bark extract against androgen-mediated oxidative stress in male Wistar rats has been studied.

2.1.2 VH Bhaskar, et al., (2009): The ethanol and aqueous extracts of the dried stem bark of the plant Crataeva nurvala Buch-Hum (Capparidaceae) have been found to possess significant anti-fertility effects in rats. Both ethanol and aqueous extracts exhibited partial and complete resorption of implants at 300 and 600 mg/kg b.wt dose levels, respectively. In estrogenic activity study, both the extracts increased uterine weight and caused opening and cornification of vagina in immature rats. The present work justifies its effectiveness in preventing pregnancy in all rats at dose levels

2.1.3 Shumaia Parvin et al,. (2011): Crude rectified spirit extract of the stem bark of Crataeva nurvala was taken under chemical investigation. The chloroform fraction yielded total four compounds namely betulinic acid (1), lupeol (2) and β-sitosterol (3) and stigmasterol (4). The structure of the isolated compounds was established by extensive analyses of 1H NMR spectroscopy and compared the spectral data, melting points with the authentic specimen. The results indicate that the stem bark of Crataeva nurvala provide a rich source of triterpenoids and steroids.

2.1.4 Sanjay Saxena et al., (2008): rataeva nurvala Buch. Ham. (Capparaceae) is a high- value medicinal tree that grows almost all over India, especially in the semiarid regions. Medicinal usage has been reported in traditional systems of medicine, such as Ayurveda and Unani, wherein the plant is frequently preferred in the treatment of urinary disorders that reoccur owing to development of antibiotic resistance by the infecting organism. C. nurvala has also been used in the treatment of prostate enlargement and bladder sensitivity. The plant is known to relieve, prevent, and promote the discharge of kidney stones. Lupeol, a pentacyclic triterpene isolated from the root bark, has been shown to significantly minimize the deposition of stone-forming constituents in kidneys. Investigations have also indicated the plant has anti-arthritic, hepatoprotective, and cardio-protective actions

2.2 Literature related to activity

2.2.1 Wei-Ling Guo et al. (2019): This study aimed to investigate the hypoglycemic and hypolipidemic activities of polysaccharides from Grifolafrondosa (GFP) in diabetic mice induced by high-fat diet (HFD) and streptozotocin (STZ). Results showed that oral administration of GFP markedly reduced the serum levels of fasting blood glucose (FBG), oral glucose tolerance (OGT), cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C), and significantly decreased the hepatic levels of TC, TG and free fatty acids (FFA). Meanwhile, high-dose of GFP supplementation (900 mg/kg day) also showed powerful effects on moderating the composition of intestinal microflora in diabetic mice, especially altering the functionally relevant intestinal microbial phylotypes. Spearman's correlation network analysis revealed that key microbial phylotypes responding to GFP intervention were strongly correlated with the glucose and lipid metabolic disorders associated parameters. Moreover, GFP treatment regulated mRNA expression levels of the genes responsible for hepatic glucose and lipid metabolism. These findings demonstrated that GFP could prevent hyperglycemia and hyperlipidemia in diabetic mice by altering gut microbiota and regulating hepatic glycolipid metabolism related genes, and therefore could be used as potential functional food ingredients for the prevention or treatment of hyperglycemia and hyperlipidemia.

2.2.2 Maria SorhedeWinzell et al. (2004): This study characterizes the high-fat diet–fed mouse as a model for impaired glucose tolerance (IGT) and type 2 diabetes. Female C57BL/6J mice were fed a high-fat diet (58% energy by fat) or a normal diet (11% fat). Body weight was higher in mice fed the high-fat diet already after the first week, due to higher dietary

intake in combination with lower metabolic efficiency. Circulating glucose increased after 1 week on high-fat diet and remained elevated at a level of 1 mmol/l throughout the 12-month study period. In contrast, circulating insulin increased progressively by time. Intravenous glucose challenge revealed a severely compromised insulin response in association with marked glucose intolerance already after 1 week. We conclude that the high-fat diet–fed C57BL/6J mouse model is a robust model for IGT and early type 2 diabetes, which may be used for studies on pathophysiology and development of new treatment. Diabetes 53 (Suppl. 3): S215–S219, 2004.

2.2.3 Amel M. Soliman et al. (2016): Antioxidant therapy has been thought to be effectual for the prevention and treatment of various diseases including diabetes. Therefore, the present study was designed to investigate the potency of Paracentrotuslividus extract (PLE) for alleviating the complications that resulted after induction of the diabetic rat models (T1DM and T2DM) using high fat diet (HFD)/streptozotocin (STZ). Thirty-six male Wistar albino rats were assigned into normal control, T1DM and T2DM untreated, and PLE treated diabetic rat groups. Induction of T1DM was performed by streptozotocin injection (60 mg/kg of dissolved in sodium citrate buffer, 0.1 mol/L, i.p). T2DM induction through 4 weeks of high fat diet (HFD) intervention was followed by a single low dosage of STZ (30 mg/kg dissolved in 0.1 mol/L citrate buffer at pH 4.5, i.p). Both diabetic rat models showed a significant increase in serum; levels of fasting glucose, total protein, bilirubin, activities of arginase, transaminases (AST and ALT), alkaline phosphatase (ALP), c glutamyl transferase (GGT), lipid profile parameters, and liver malondialdehyde (MDA). However, T1DM and T2DM rats have decreased levels of serum insulin, and liver glucose 6 phosphate dehydrogenase (G6PD), glutathione reduced (GSH), nitric oxide (NO), and antioxidant enzymes.

2.2.4 Mahmoud Nassar et al (2021): The aim is to cover most of the current evidence on the mutual effect of diabetes & COVID-19 infection on each other and the management of the COVID-19 patients with diabetes. they utilized databases to review the current evidence related to diabetes mellitus and COVID19. They discussed the most recent evidence of diabetes milieus and COVID-19 regarding risk factors, management, complications, and telemedicine. Diabetes mellitus is associated with a significant risk of complications, extended hospital stays, and mortality in COVID-19 infected patients.

2.2.5 Ravi Prakash Upadhyay et al. (2013): To estimate the burden of diabetes mellitus and pre-diabetes in tribal populations of India. The authors reviewed studies from 2000 to 2011 that documented the prevalence of diabetes mellitus in various tribal populations of India. The search was performed using electronic and manual methods. Meta-analysis of data on point prevalence was performed. A total of seven studies were retrieved. The prevalence of diabetes mellitus ranged from 0.7% to 10.1%. The final estimate of diabetes prevalence obtained after pooling of data from individual studies, was 5.9% (95% CI; 3.1–9.5%). The prevalence for impaired fasting glucose (IFG) varied from 5.1% to 13.5% and impaired glucose tolerance (IGT), from 6.6% to12.9%. Chronic disease research in tribal populations is limited. The reported prevalence of IFG/IGT was higher than the prevalence of diabetes and this observation could be suggestive of a potential increase in diabetes in the coming years. Given that lifestyle changes have occurred in the tribal populations, there is a need to synthesize evidence(s) relating to diabetes and other chronic diseases in these marginalized populations and inform policy makers.

3. REASEARCH ENVISAGED

OBJECTIVE:

3.1Objective:

- Create a plant extract phytochemical screening system.

- To conduct an acute oral toxicity study in accordance with OECD guideline 425 in order to determine dosage and assess drug safety.
- To assess Crataeva nurvala bark and leaf extract's antihyperlipidemic properties in a model induced by the HFHS diet.

3.2 Rationel:

The plant is, according to preliminary phytochemical screening. abundant in alkaloids, glucosilinates, flavonoids, triperpenoids, saponins, and phytosterols. The stem bark has yielded phytoconstituents such as diosgenin, cadabicinem diacetate, friedelin, ceryl alcohol, lupeol and its acetate, and betulinic acid. Fruits contain cetyl and ceryl alcohol, triacontanol, and glucocapparin. L-stachydrine, dodecanoic anhydride, methyl pentacosanoate, kaemferol-0-α-D-glucoside, and quercitin-3-0-α-D-glucoside were all detected in the leaves. Rutin, quercitin, varunol, and β-sitosterol are all found in root bark. Cadabicine and cadabicine diacetate (Alkaoids) are detected in the isolated substance. The work offers useful information about animal safety and effectiveness, and it can be expanded to include clinical research.

We tried to look into the traditional polyherbal formulation for their antihyperlipidemic effects in the HFHS diet-induced model in light of the literature review mentioned above.

4. PLAN OF WORK

- Preliminary Work
- Literature Review.
- Plant Collection.
- Plant Authentication.
- Drying & Size Reduction of Plant Material.
- Preparation of Crude Extract by Extraction Method.
- Phytochemical Screening of Crude Extract.
- In vivo toxicity syudy
- Acute Oral Toxicity Study (OECD 425 guideline).
- In Vivo anti -obesity and anti-hyperlipedimic activity
- HFHS diet induced Antihyperlipidemic Model.
- ➢ Evaluation parameters
- Effects on body weight (mg/dl)
- Effects on Lipid profile (mg/dl)

5. PLANT PROFILE

5.1 Crataeva nurvala

About 70 species, mostly found in the warmer (tropical) regions of the world, make up the genus Crataeva, which bears the name of the Greek botanist Crataevas. In India, C. nurvala has the highest level of biodiversity among them. The following is C. nurvala's taxonomical categorization.

Kingdom: Plantae
Division: Magnoliophyta
Phylum: Tracheophyta
Class: Magnoliospida
Order: Brassicales
Class: Magnoliopsida Brongniart
Family: Capparidaceae A.L. de J

5.2 Crataeva nurvala's phytoconstituents The plant is abundant in triperpenoids, saponins, flavonoids, phytosterols, alkaloids, and glucosilinates, according to preliminary phytochemical screening. The stem bark has yielded phytoconstituents such as diosgenin, cadabicine diacetate, friedelin, ceryl alcohol, lupeol and its acetate, and betulinic acid. Fruits contain cetyl and ceryl alcohol, triacontanol, and glucocapparin. L-stachydrine, dodecanoic anhydride, methyl pentacosanoate, kaemferol-0-α-D-glucoside, and quercitin-3-0-α-D-glucoside were all detected in the leaves. Rutin, quercitin, varunol, and β-sitosterol are all found in root bark.

5.3 Botanical Descriptions

A little tree with many branches is Crataeva nurvala Ham. The tree reaches a height of 25 to 30 meters.

Leaves: Deciduous, 3-foliate; leaflets 5-15 by 3.8-6.3 cm; petioles 3.8-7.6 cm long. whole, glabrous on both surfaces, pale underneath, reticulately veined, lanceolate or obovate, acute or acuminate, attenuate at the base, with lateral leaflets oblique at the base; petiolules 6- 9 mm long.

Flowers: Numerous greenish white terminal corymbs with pedicels ranging from 2.4 to 4.4 cm. Glabrous and long-stout. Petaloid, tiny, oval, sharp, and distant sepals. The claw is up to 6 mm long and extremely slender, while the petals (including the claw) measure almost 2.5 by 0.9 cm. spreading stamens that are longer than the petals. Gynophores are smooth, terete, and about 5 cm long. ellipsoid ovary; flat stigma.

Fruits: A berry on the thickened gynophores that is globose or ovoid, woody, smooth, or scurfy. Brown

seeds embedded in pulp that is almost smooth. The gynophores are violet, the stamen filaments are purple or white when young, and the sepals are green when young and yellow or pale pink when old.

5.4 Distribution: Wild or cultivated, found almost everywhere in India and Burma. found in the sub-Himalayan region in arid deep boulder formations as well as frequently beside streams.

5.5 Medicinal uses

Parts medicinally used are bark, leaves, root & flower.

Bark: Vata, well in strangury, and biliousness are eliminated by the bark's stomachic, laxative, antilithic, vesicant, anthelmintic, detergent, bechic, and expectorant properties. It also has a hot, bitter, and then sweet, sharp taste that is easy to digest. The bark eliminates illnesses or urinary organs, increases appetite, and reduces bile and phlegm emissions. Additionally, it has sedative, antipyretic, demulcent, alterative, and tonic properties. Some moderate kinds of skin diseases, fever, and urinary problems can benefit from the bark.

Leaves: The leaves are used as a rubefacient on the outside and as a febrifuge and tonic on the inside. They are also stomachic and tonic.

Root: The leaves are used internally as a febrifuge and tonic, and topically as a rubefacient. They are also stomachic and tonic.

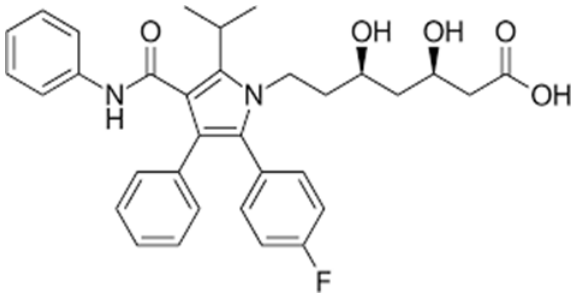
Flowers: The blooms have cholagogue and astringent properties.

6. DRUG PROFILE

6.1 Atorvastatin

Atorvastatin is a member of the class of medications known as HMG CoA reductase inhibitors, or "statins."When taken in conjunction with a healthy diet, atorvastatin helps reduce blood levels of "bad" cholesterol (low-density lipoprotein, or LDL), raise levels of "good" cholesterol (high-density lipoprotein, or HDL), and bring down blood fat (triglycerides).

6.2 Mechanism of action



3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is competitively inhibited by atorvastatin. Statin drugs reduce the liver's synthesis of cholesterol by blocking the conversion of HMG-CoA to mevalonate. Additionally, atorvastatin increases the quantity of LDL receptors on the hepatic cell surface. Atorvastatin has been demonstrated to decrease total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), apolfipoprotein B (apo B), very-low-density lipoprotein (VLDL-C), and triglycerides (TGs) while raising high-density lipoprotein cholesterol (HDL-C) in patients with homozygous or heterozygous familial hypercholesterolemia, mixed dyslipidemia, isolated hypertriglyceridemia, or no familial hypercholesterolemia. It has been demonstrated that atorvastatin lowers intermediate-density lipoprotein (IDL-C) in patients with dysbetalipoproteinemia.

Figure No.1. Structure of Atrovastatin

6.3 Side effects of Atorvastatin

- Symptoms of the digestive system, like diarrhea
- Signs of cold, such a runny or congested nose
- Pain in the joints
- Sleeplessness
- Infection of the urinary tract
- queasy
- A decrease in appetite
- Symptoms of ingestion, such as pain or discomfort in the stomach
- A rise in transaminases
- Muscle contractions that may or may not be painful
- Muscle, ligament, tendon, bone, and joint discomfort is known as musculoskeletal pain. Pain in the muscles
- Pain in the legs
- Pain in the throat and mouth

6.4 Contraindication

- Hepatitis, jaundice, cholestasis, and hepatic encephalopathy are signs of active liver disease.
- Unexplained elevations in AST or ALT levels
- Pregnancy: By altering blood cholesterol and triglyceride levels, which are critical for fetal development, atorvastatin may harm the fetus.
- Breastfeeding: While atorvastatin has not been extensively investigated, small levels of other statin drugs have been shown to transfer into breast milk. Atorvastatin is not considered breastfeeding compatible due to the possibility of interfering with a nursing infant's lipid metabolism.
- Significantly raised CPK values or if a myopathy is identified or suspected after starting atorvastatin dosage. Rhabdomyolysis is a rare but potentially dangerous side effect of atorvastatin that can result in acute kidney damage from myoglobinuria. in the event that rhabdomyolysis is identified or suspected.

6.5 Therapeutic Uses:

In patients with type 2 diabetes, coronary heart disease, or other risk factors, atorvastatin is used to treat excessive cholesterol and reduce the risk of stroke, heart attack, or other heart problems.

500 MG/KG of LD and ED 50-ATROVASTATI

7. MATERIALS AND METHODS

7.1 Materials

7.1.1. Test Drug-Crataeva nurvala

- Standard Drug- Atrovastatin
- Animals Used- For In-Vivo Study
- No. of Rats - 42
- Sex - Male / Female (either)
- Strain - Albino Wistar Rats
- Weight - 180- 250gm

7.1.2. Source- Ravishankar College of Pharmacy, Bhopal (M.P.)

- (Proposal no RCOP/IAEC/DEC/2024/13)

7.1.3. Housing condition:

In separate cages 12-12hour light and dark cycle

- Relative Humidity - 40-60%
- Temperature - 25˚C (±2˚C)
- Diet- Standard Food pellets consumed by Rats

7.1.4 Other requirments:

- Glassware's- Beaker, Glass rod, Measuring Cylinder, Petri-dish, Test-tube, Pipette, Micropipette.
- Chemicals- Ethanol, Chloroform, CMC, Sodium Hydroxide, Hydrochloric Acid, Sulphuric acid, Benzene, Iodine, Lead Acetate, Magnesium Turnings, Nitric Acid, Gelatin, Ferric Chloride, Sodium Chloride.
- Other- Oral feeding Neddle,Cappillary tube, Cotton, Markers, Eppendrof tube, Gloves, Marker pen, Syringe-5 ml etc.

7.1.5 Instrument:

- Soxhlet apparatus (KHERA).

7.2 Method:

7.2.1. Preliminary work (Selection of Plant)

After compiling enough data from numerous publications and journals, it was determined that there is room to investigate other pharmacological properties in the bark of the plant Crataeva nurvala; as a result, it was chosen for additional research.

7.2.2. Collection and Authentication of Plant Material

- Collection – Vindhya Herbal Garden.
- Authentication of Plant- Identification and authenticated by Dr Saba Naaz Head of the Department Botany at the Safia college of science, Bhopal (M.P.). The plant part specimen was submitted as herbarium.

7.2.3. Plant material drying, shrinking, and storing

- Drying Dried under the shade.
- Size reduction and filtration- Test material shall be pulverized to coarse powder with the help of mixer grinder and the coarse powder obtained have to passed through sieve No. 20 to maintain uniformity.
- Packging to maintain uniformity and packed into airtight container and stored in cool and dry place. This material was used for the further study.

7.2.3. Preparation of Crataeva nurvala bark (L.) extract

The Soxhlet extraction method was used to extract Crataeva nurvala. (using the conventional approach). The process of extraction is depicted in figure:

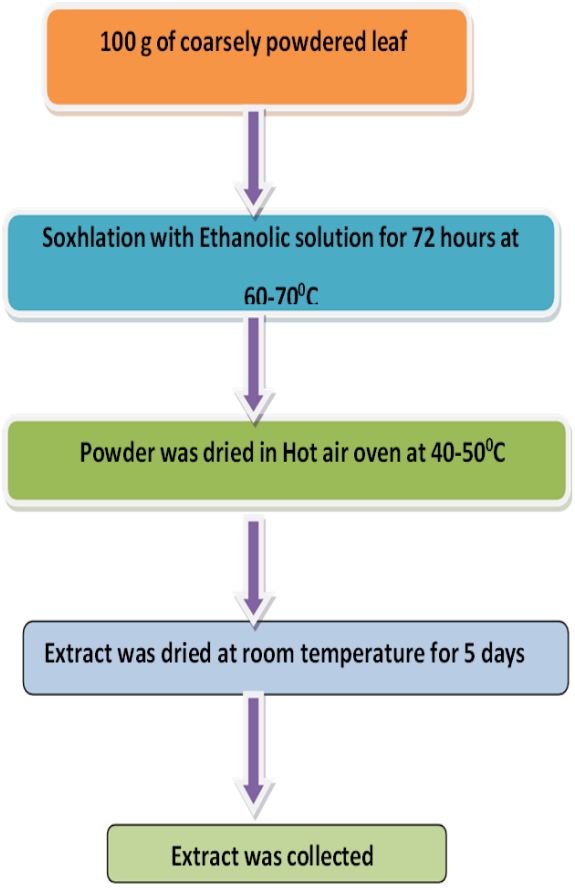


Figure no. 02: Extraction Process (Soxhlet apparatus)

7.2.5 Phytochemical Analysis Of Crude Extracts (Khandwelkre1997)

In order to identify common chemical elements such as alkaloids, glycosides, carbohydrates, phytosterols, saponins, tannin, flavonoids, and protein, among others, the crude extracts of plants that were obtained by solvent extraction were put through a number of quality tests.

7.2.5.1 Test for alkaloids:

- Dragendroff's test: One milliliter of extract and one milliliter of the potassium bismuth iodide solution, or Dragendroff's reagent, were added to the test tube. The presence of alkaloids was revealed by the appearance of an orange-red precipitate.
- Mayer's test: One milliliter of extract and one milliliter of Mayer's reagent (solution of potassium mercuric iodide) were put into the test tube. Alkaloids were present when a whitish yellow or cream-colored precipitate formed.
- Wagner's test: One milliliter of extract and one milliliter of Wagner's reagent (iodine potassium iodide solution) were put into the test tube. A reddish-brown precipitate suggested that alkaloids were present.

7.2.5.2 Test for carbohydrates, gums and mucilage:

- Benedict's test: 1 ml of extract solution was added to 5 ml of Benedict's reagent, which was then heated for 2 minutes and allowed to cool. The presence of sugars was revealed by the formation of crimson precipitate.
- Molisch's test: A reddish violet ring at the junction of the two layers indicated the presence of carbohydrates. A small fraction of extract was taken in ethanol separately, and a few drops of a 20% w/v solution of α-napthol in ethanol (90%) were added to it. After thoroughly shaking, approximately 1 ml of concentrated sulfuric acid was allowed to flow carefully by the side of the test tube.
- Fehling's test: The presence of carbohydrates was shown by the formation of a brick-red precipitate after the extract was heated with dilute HCL, neutralized with NaOH, and Fehling's solutions A and B were added.
- Test with 95% alcohol: When 95% alcohol added to the extract, gums get precipitated out. The precipitate is insoluble in alcohol.
- Ruthenium red test: In this test, the mucilage is stained red by 0.08 grams of ruthenium red diluted in 10 milliliters of a 10% lead acetate solution.

7.2.5.3 Test for glycosides:

- Killer-killani test: After dissolving two milliliters of the extract in glacial acetic acid, one drop of 5% FeCl3 and concentrated H2SO4 were added. The presence of glycosides was indicated by the upper layer appearing bluish green and the junction of the two liquid layers turning reddish brown.

- Baljet's test: When 1 milliliter of sodium picrate solution was added to 1 milliliter of the test extract, the presence of glycoside was shown by the color turning from yellow to orange.
- Foam test: When 0.5g of extract is agitated vigorously with water, a layer of foam forms. The presence of glycosides is indicated.

7.2.5.4. Test for flavonoids:

- Shinoda test: After a few minutes, the test solution turns pink scarlet, crimson red, or occasionally green to blue when a few magnesium turnings and powerful hydrochloric acid are added drop by drop.

7.2.5.5. Test for phenolic compounds and tannis:

- Test with lead acetate: Tannins get precipitate with lead acetate.
- Test with ferric chloride: Phenols were often identified by precipitating them with a 5% w/v solution of ferric chloride in 90% alcohol.
- Test with gelatin solution: An aqueous solution of gelatin (1%) and sodium chloride (10%) was added to a solution of tannins (0.5–1%). The compounds are confirmed by a white, buff precipitate.

7.2.5.6. Test for Proteins:

- Biuret test: Before adding the 1 ml of extract, mix in 1 ml of 40% sodium hydroxide solution and 2 drops of 1% $CuSO_4$ solution until a blue tint appears. Protein is present when a pinkish or purple-violet hue forms.

7.2.5.7. Test for Saponin:

- Froth test: One milliliter of distilled water was added to a small portion of the extract, which was then shaken. The presence of saponins was detected by the appearance of a distinctive foam structure. Direct tests were conducted on alcoholic and aqueous extracts.
- Foam test: Two milliliters of distilled water were mixed with a small portion of the extract. Each was shaken after a tiny amount of sodium carbonate was added. The presence of saponins was detected by the distinctive foam formation. Direct tests were conducted on alcoholic and aqueous extracts.

7.2.5.8. Test for Steroids:

- Salkowski test: After dissolving the extract in chloroform, an equivalent volume of concentrated H_2SO_4 was added. The steroidal components in the examined extract are represented by the formation of a bluish red to cherry color in the chloroform layer and green fluorescence in the acid layer.
- Liebermann-Burchard test: A tiny amount of the extract was combined with roughly 1 milliliter of acetic anhydride and warmed until it dissolved. A few drops of strong sulfuric acid were introduced to each case by the test tube's walls after the contents had cooled. The presence of sterols was indicated by a blue appearance.

7.2.5.9. Experimental work:

Animals: For the investigation, adult Wistar rats weighing 180–250 g were employed. Rats for the experiment were acquired from the Ravishankar College of Pharmacy in Bhopal, Madhya Pradesh. The animals were kept in controlled environments with 12 hours of light-dark cycles, a temperature of 23 ± 2°C, and a humidity of 50 ±5%. Prior to the trial, all of the animals were acclimated for seven days. The animals were kept separately in sterile polypropylene cages with sterile husk bedding after being randomly assigned to experimental and control groups. They were fed a basal diet of free-assessed standard pellets and unlimited water. For 48 hours before the experimental protocol, the animals were acclimated to the laboratory environment in order to reduce any non-specific stress. The Institutional Animal Ethical Committee (IAEC) of Ravishankar College of Pharmacy in Bhopal, Madhya Pradesh, accepted all of the experiments in accordance with the rules set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India. 1733/PO/Ere/S/13/CPCSEA is the approval number.

Induction of Hyperlipidemia (HFHS standard model to be proposed):

In terms of calories, the regular diet included 11.4% fat, 62.8% carbohydrate, and 25.8% protein (total 12.6 kJ/g), while the high-fat diet had 58% fat from lard, 25.6% carbohydrate, and 16.4% protein (total 23.4 kJ/g). Blood samples were obtained from the intraorbital retrobulbar plexus of nonfasted

anesthetized mice at specified intervals, and food consumption and body weight were recorded once a week. (Winzell et al., Maria Sorhede 2004)

Experimental procedure:

The animals in the HFHS Diet model were split up into five groups, with six animals in each group. The following groups of animals were formed:Group I is the normal group, which follows a normal pellet diet. Positive group (ND + HFHSD) is group II.Group III: HFHSD + ND + Atorvastatin Calcium (2.1 mg/kg, p.o. body weight) (Standard Group) Group IV: HFHSD + ND + Extract (300 mg/kg, p.o. body weight) (Test Group) Every treatment was administered for 42 days. To increase the rate of absorption, the animals were fasted for two hours both before and after the treatment. Body weights, average feed intake, blood glucose, total cholesterol, HDL, LDL, VLDL, and triglyceride levels were among the parameters examined for this test.

Preparation of Dose:

Before being given to the rats orally, the test extract of Crataeva nurvala bark was dissolved in 1%CMC, a suspending agent. Rats were given the standard medication (atorvastatin) orally after it had been dissolved in a suspending agent (1% CMC).

Toxicity study: In accordance with the OECD (Organization for Economic Cooperation and Development) 425 Guidelines, the acute oral toxicity test of methanolic leaf powder extract was conducted before the efficacy investigation.Murraya Paniculata Leaf powder (TCLP) was given to female Albino Swiss mice at a low dose of 2000 mg/kg body weight.Over the course of 14 days, the treated animals' health, clinical signs, and symptoms were monitored. Even at a larger dosage of 2000 mg/kg body weight, there was no mortality.

8. RESULT

8.1 Morphology:

Table no. 1: Morphological characteristics of bark of Crataeva nurvala (L)

S. No.	Character	Observation
1	Color	Greenish
2	Odor	None
3	Taste	Characteristic
4	Size	10-20 cm. Length

8.2 consistancy and color

Table no. 2: Consistency and color of Crataeva nurvala (L) bark extract

Extract	Color	Consistency
Hydro-Alcoholic	Dark Green	Semi solid

8.3 Practical & Percentage Yield:

Table no. 3: Percentage yield of Crataeva nurvala (L) bark extract

S. No	Extracts	Yield (gm)	Percentage Yield
1	Hydro-alcoholic	12.801	14.94%

8.4 Screening of powder:

Table no. 4: Physiochemical analysis of powder of Crataeva nurvala (L) bark extract

8.5 Phytochemical screening:

S. No.	Parameters	Observation (%)
1	Loss on drying	1.05
2	Total cash value	4.89
3	Acid insoluble ash value	1.00
4	Water soluble ash value	0.97
5	Foaming index	0.8 cm

The hydro-alcoholic extract of Crataeva nurvala (L) bark contains proteins, alkaloids, carbohydrates, flavonoids, glycosides, and saponins.

Table no. 5: Phytochemical screening of hydro - alcoholic extract of Crataeva nurvala (L)bark extract

S. No.	Identification Test	Test name	Results
1	Alkaloids	Mayer's test	-
		Dragendroff's test	+
		Wagner's test	+
2	Glycosides	Killer-killani test	-
3	Carbohydrates	Molisch's test	+
		Fehling test	+
4	Tannins & Phenols	Gelatin test	+
		Ferric chloride test	+
5	Flavonoids	Shinoda test	-
		Alkaline reagent test	-

6	Steroids	nm-Burchard test	+
		Salkowski test	+
7	Saponins	Foam test	-
8	Protein	Xanthoprotic	+
9	Gums & Musilage	With 95% Elcohol	-

(+) = Present, (–) = Absent

8.6 Acute Toxicity Studies (LD50): No animal died, showed any behavioral changes, or displayed any toxicity after receiving a single dose of HACD (2000 mg/kg p.o.) in either the Phase I or Phase II procedures. For this investigation, a dosage of 300 mg/kg was chosen.

Table no. 6: Results of Acute oral toxicity study of HACN

Group name	Animal mark	Dose mg/kg	Body weight (gm)			Observation	Mortality (If any)
			1 day	7 days	14 day		
Control	H	Normal saline (0.91%)	156	149	146	No sign of toxicity & all animals Survived.	No mortality occurs. .
	B		145	140	135		
	T		125	123	122		
Test	HT	2000 mg/kg of Extract (Once dosing at start of acute oral toxicity study)	209	210	205		
	BT		200	195	190		
	NM		175	165	155		

8.7 Evaluation parameter:

8.7.1 Effect on Feed (gm) and water (ml) intake:

Table no. 7: Total feed (gm) and water (ml) intake:

Group	Pellet Diet (gm)	HFHS Diet (gm)	Water intake (ml)
NC	93.38 ± 17. 53	0	286.67 ± 76.60
PC	56.09 ± 5.70*	125.73 ± 18.41	305.67 ± 86.98
STD	57. 08 ± 12.13*	149.16 ± 9.99	302.00 ± 51.57
TEST	71.40 ± 12.78	164.28 ± 10.82	416.33 ± 20.88

All values are Mean ± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group. (Following Repeated measures ANOVA (parametric methods, using Dunnett Test.)

8.7.2 Effect on Body Weight(gm):

Table no. 8: Body weight (gm)

Group	0^{th} day	7^{th} day	14^{th} day	21^{th}day
NC	123.83 ± 2.24	138.67 ± 3.92	130.17 ± 3.53	130 ± 6.35
PC	139.17 ± 4.28	141 ± 4.20	143.33 ± 3.79	147.17 ± 3.61

STD	145.33 ± 8.17	147.83 ± 8.58	145.17 ± 8.02	141.17 ± 7.68
Test	177 ± 13.92*	183.67 ± 13.42*	180.67 ± 13.37*	172.5 ± 10.43

All values are Mean± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group. (Following Repeated measures ANOVA (parametric methods, using Dunnett Test.)

8.7.3. Effect on HDL level (mg/dl):
Table no. 9: HDL level (mg/dl)

Group	0^{th} day	7^{th} day	14^{th} day	21^{th}day
NC	48.67 ± 6.73	58.17 ± 5.59	49.83 ± 5.90	48.33 ± 3.99
PC	49 ± 1.48	51 ± 4.07	48.83 ± 2.85	44.33 ± 3.73
STD	43.17 ± 3.40	50.5 ± 5.47	53 ± 6.87	49 ± 4.25
Test	48.5 ± 4.60	62 ± 4.77	55.33 ± 6.35	53.5 ± 7.72

All values are Mean± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group. (Following Repeated measures ANOVA (parametric methods, using Dunnett Test.)

8.7.4 Effect on LDL Level (mg/dl):
Table no. 10: LDL level (mg/dl)

Group	LDL level (mg/dl)			
	0^{th} day	7^{th} day	14^{th} day	21^{th}day
NC	73.33 ± 7.92*	66.5 ± 5.64	64.17 ± 4.79	58 ± 5.46*
PC	46.83 ± 2.47	58.17 ± 2.23	70.17 ± 2.30	76.67 ± 3.33
STD	39.33 ± 2.32	50.67 ± 2.57	51.5 ± 4.25*	55 ± 4.67**
TEST	43.5 ± 5.97	48.17 ± 4.91	48.5 ± 4.82**	56 ± 5.89**

All values are Mean± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group. (Following Repeated measures ANOVA (parametric methods, using Dunnett Test.)

8.7.5 Effect on CHL level (mg/dl):
Table no. 11: CHL level (mg/dl)

Group	CHL level (mg/dl)			
	0^{th} day	7^{th} day	14^{th} day	21^{th}day
NC	139.5 ± 7.75	149.5 ± 4.89	142.67 ± 7.13*	149.5 ± 4.68**
PC	143.67 ± 6.86	155 ± 5.05	173.83 ± 5.22	196.83 ± 3.33
STD	144.33 ± 10.10	156.67 ± 8.85	173.33 ± 8.95	181.83 ± 3.87
TEST	154.33 ± 9.74	171.5 ± 9.40	175.67 ± 5.41	185 ± 4.24

All values are Mean± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group. (Following Repeated measures ANOVA (parametric methods, using Dunnett Test.)

8.7.6. Effect on Blood glucose level (mg/dl):

Table no. 12: Blood glucose level (mg/dl)

Group	0^{th} day	7^{th} day	14^{th} day	21^{th}day
NC	150.50 ± 6.65**	147.83 ± 5.94**	142.67 ± 3.54**	141.67 ± 3.77*
PC	222.33 ± 17.17	239.33 ± 8.93	205.50 ± 14.48	181.5 ± 6.61
STD	234.33 ± 11.34	182 ± 6.76**	170 ± 10.80	145.33 ± 9.91
Test	233.83 ± 6.43	217.17 ± 9.86	177.83 ± 10.75	133.67 ± 4.60

All values are Mean± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group. (Following Repeated measures ANOVA (parametric methods, using Dunnett Test.)

Evalutaion parameter: When compared to the vehicle-treated group and the control group (using the Dunnett Test, repeated measures ANOVA, and parametric techniques), all data are Mean ± SEM, n=06, *P<0.05, **P<0.01.

Discussion: One of the primary factors contributing to excessive fat mass buildup, or obesity, which in turn promotes other metabolic disorders including dyslipidemia, is a high-fat diet.[14] Consequently, a high-fat diet (HFD) model was employed to generate human-like dyslipidemia. Body weight gain and fat accumulation are the main markers of the slow development of obesity. Adiposity increased as a result of the animals' HFD diet, and this in turn led to an increase in the mass of fat cells. As a result, body weight increased overall.

The ingestion of a diet high in energy, such as lard, which is saturated fat and deposits in different body fat pads, and a lower energy expenditure than in animals fed NPD may be the causes of the higher body weight observed in HFD rats [14].[15] Nonetheless, there was a notable drop in body weight and fat mass following piperine administration, demonstrating its anti-obesity properties. The most significant link between obesity and coronary artery disease is dyslipidemia.[16] Increased small dense LDL composition, decreased HDL levels, and elevated triglycerides are the most prevalent features of dyslipidemia associated with obesity.[16] Increased dietary cholesterol absorption from the small intestine after consuming an HFD may be the cause of hypercholesterolemia. [17, 18]

Pharmacognocal and phytochemical screening was carried out following the extraction. The phytoconstituents included in Cordia dichotoma leaf extract, including alkaloids, carbohydrates, steroids, proteins, and tannins, may be responsible for the extract's ability to decrease cholesterol.

Even at the highest tested dose level of 2000 mg/kg per mouth, no death was noted until the end of 24 hours in the acute oral toxicity trial, and no behavioral abnormalities were noticed until the first 4 hours. It was regarded as the highest safe dosage. Measurements of the study included blood glucose level (mg/dl), lipid profile HDL, LDL, CHL level (mg/dl), daily feed consumption (gram), water intake (ml), and body weight (gm).

9. CONCLUSION

In conclusion, we can state that the bark of Crataeva nurvala (L|) has antihyperlipidemic activity in a rat model generated by the HFHS diet and that it greatly improves the lipid profile levels in experimental rats. The blood glucose level and serum lipid profile, including total cholesterol, significantly decreased, according to the results. LDL and raising blood HDL levels, which may help treat hyperlipidemia as it is described in conventional medicine. To identify the phytochemicals causing the hyperlipidemic action, more research is necessary.