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EVALUATION OF HYPOLIPIDAEMIC EFFECT OF HYDROETHANOLIC EXTRACT OF LEAVES OF MUSA PARADISICA (BANANA) IN HIGH FAT DIET FED WISTAR RATS

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Abstract:

Lipid lowering effect of hydroethanolic extract of Musa paradisica leaves was evaluated in high fat diet induced hyperlipidemic models of wistar albino rats. Test extract inhibited the elevation in serum cholesterol and triglyceride levels on high fat diet administration rats. The extract at the same dose level significantly attenuated the elevated serum total cholesterol and triglycerides with an increase in high-density lipoprotein cholesterol in high-fat diet-induced hyperlipidemic rats. The standard drug Atrovastatin showed slightly better effects. In histopathological study it was found that treatment of test extractsignificantly decrease in the dilatations in the sinusoidal capillaries of the liver, cytoplasmic fatty infiltration, and granular degeneration when compared with the high-fat diet-fed group II animals. The outcome of the study reveals the lipid lowering activity of hydroethanolic extract of Musa paradisiac in dyslipidaemic conditions by interfering with the biosynthesis of cholesterol and utilization of lipids.

Keywords: *Musa pradisica, antihyperlipidemic activity, High fat diet, HDL-C, Triglycerides.*

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INTRODUCTION:

The global health issues of obesity and high cholesterol are becoming increasingly serious and complex. The World Health Organization (WHO) has declared obesity an epidemic, with over 1.9 billion adults being overweight and 650 million of them classified as obese, highlighting the severity of the problem. Simultaneously, hyperlipidemia, marked by elevated cholesterol and triglyceride levels, has become a serious global burden, contributing to a noteworthy 4.5 million deaths in 2017, according to the Global Burden of Disease Study.

Hyperlipidemia, a medical condition characterized by elevated lipid levels in the blood, presents a substantial challenge in contemporary healthcare. This discussion aims to explore the intricate aspects surrounding hyperlipidemia, covering its origins, consequences, and potential avenues for management, while also delving into the mechanistic pathways and available drugs for treatment as shown in Fig.

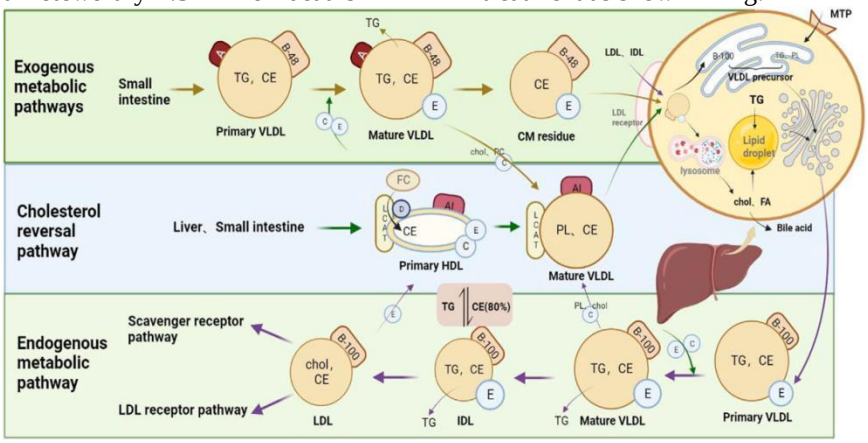

Figure: Blood Lipid Metabolism

A detailed investigation into lipid metabolism pathways reveals that the abundance of calories inherent in obesity prompts heightened synthesis and storage of triglycerides in adipose tissue. At the same time, the liver undergoes alterations in lipid processing, leading to elevated lipid levels circulating in the bloodstream. This disturbance in lipid metabolism extends beyond mere accumulation, playing a significant role in the intricate connection between obesity and hyperlipidemia[18].

Musa paradisiaca

Taxonomical Classification

Table 1: Taxonomical classification of *Musa paradisiaca*

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Sub-class	Zingiberidae
Order	Zingiberales
Family	Musaceae
Genus	*Musa*
Species	*paradisiaca*

MATERIAL & METHODS:

Drugs and Chemicals

Atrovastatin, Ethanol, Citric acid, Sodium citrate, Hydrochloric acid, NaOH and EDTA.

Collection, Identification and Authentication of plant material: The fresh leaves of Musa paradisiaca were collected from the field of Varanasi district in the month of their natural habitat. All leaves were cleaned of extraneous matter and ground into a fine powder. Plant and plant material was identifiedby comparing morphological features of plant. The herbarium of collected plant material was prepared & the taxonomical identification (authentication) of the plant is done by Botanical Survey of India, Pune, Maharashtra, India.

The collected Leaves of Musa paradisiaca L were dried in shade to avoid loss of phytoconstituents. After drying, leaves were coarsely powdered with grinding mill and kept in air tight container for use in the study. Based upon the extensive literature survey, we decide to use the 50%hydroethanolic solvent such as ethanol in water for extraction of phytoconstituent from powder of leaves of Musa paradisiaca for further study.

Extraction of plant materials

The procedure is to grind coarsely ground plant material in a paper thimble and place it in a Soxhlet apparatus. The solvent is put into the thimble and collected into the Round Bottom Flask (RBF). Extractions are carried out at elevated temperatures until the bead mark of the siphon tube is attained. The solvent vapors condense and raise the level of the solvent until all coloring material is removed. The solutions obtained are combined and the plant material isextracted using ethanol in water[59]. The dried extracts are weighed and placed in screw-capped vials and labelled for quantification.

Pharmacognostic Evaluation

In this determination of physiochemical parameters and extractive value of plant material as per the procedure given in standard official book.

Preliminary Phytochemical screening

The Musa paradisiac leaves extract was screened for the presence of alkaloids, saponins, tanins, carbohydrates, flavonoids, anthraquinones, cardiac glycosides, deoxy sugar, steroids and terpenes using standard methods as described by Harborne (1973), Sofowora (1993), Trease and Evans (1986), William, Trease, and Evans (1996).

Thin Layer Chromatography

For the chromatographic analysis of Musa paradisiaca leaf extracts using Thin Layer Chromatography (TLC), different mobile phase systems were employed based on the nature of the compounds. Alkaloids were best separated using a mixture of **Toluene: Ethyl Acetate: Ammonia in the ratio (7:3:1)**, which provides an optimal basic environment for alkaloid migration. For flavonoids, a non-polar to moderately polar solvent system comprising **Chloroform: Methanol (9:1)** was used, allowing effective resolution of flavonoid constituents. Phenolic compounds were separated using **Hexane: Ethyl Acetate (7:3)**, providing good migration and clear band separation on the TLC plate.

UV-Visible (UV-Vis) spectroscopic analysis

Musa paradisiaca leaf extracts in methanol and chloroform were analyzed using UV-visible spectroscopy. The same solvents were used to dilute the samples to a ratio of 1:10. Using a Perkin Elmer Spectrophotometer, the extracts were scanned in the 200–800 nm wavelength range, and distinctive peaks were found.

Pharmacological Study

The study used a healthy adult 150–200 g Wistar albino rats of either sex were employed that were received from animal house of SHEAT College of pharmacy, Varanasi. The rats were kept in rooms with standard room temperature (23 ± 1oC) and relative humidity of 67%. They also received unrestricted access to water and standard rodent food pellets. One day before to the experiment and every day for at least one hour before the experiment, they were acclimated to the laboratory conditions. Every experiment was carried out between 09.00 and 17.00 hours. All research was carried out in compliance with the guideline set forth by government of India committee for the purpose of control and supervision on experiments on animal (CCSEA), and prior approvals were obtained from the IAEC committee of SHEAT College of Pharmacy, Varanasi.

Acute oral toxicity of Musa paradisica leaves extract

Acute toxicity studies for hydroethanolic leaf extract was conducted according to OECD guideline 420 using wistar albino rats.A healthy adult 150–200 g wistar albino rats of either sex were employed. A single oral dose of the plant extract at a dosage of 2000 mg/kg body weight was given to fasted rats (n = 6 per group). The animals were observed one-on-one for the first half hour, then at regular intervals for the next 24 hours (with special attention to the first 4 hours), and finally daily for the next 14 days to search for signs of toxicity (such as changes in skin, fur, eyes, respiration, or behavioral patterns) and mortality.

High fat diet-induced hyperlipidemia

The animals were split up into four groups, each consisting of six animals. Group 5: HFD induces hyperlipidemia and is treated with the standard medication atorvastatin (100 mg/kg b. w.), Group 4: HFD induces hyperlipidemia and is treated with High dose of extract (400 mg/kg b. w.), Group 1: Control, Group 2: HFD treatment, Group 3: HFD induces hyperlipidemia and is treated with Low dose of extract (200 mg/kg b. w.). All the other groups except Normal Control were administered HFD followed by Respective treatment. Induction of hypercholesterolemia in rats was confirmed on the 10th day by elevated levels of cholesterol in all groups except Normal Rats. The HFD was discontinued, and the respective treatments were continued till Day 28.

During the study, weekly body weight and food consumption were monitored. At every 7-day interval Glucose, Cholesterol, HDL Cholesterol and Triglyceride were monitored from the serum of all groups and results were noted.

Histopathology

For histological investigations, a part of the liver tissue was preserved in 10% buffered neutral formalin. Tissues were embedded in paraffin after fixation and solid slices were cut to a thickness of 4-5 mm and stained with hematoxylin and eosin. Photomicrographs and a light microscope were used to analyze the slices.

Statistical Analysis

The results were reported as Mean+SEM using Graph Pad Prism version 5.0 and subjected to a one-way ANOVA and comparison test for statistical significance.

RESULTS:

Authentication and Pharmacognostic Evaluation

Table : Pharmacognostic evaluation of Musa paradisica leaves

Sr. No.	Parameters	Results
Physicochemical parameter		
1	Colour	Dark green,
2	Shape	Oblong
3	Texture	Smooth
4	Odour	Mild
5	Taste	Bitter,Earthy
6	Arrangement	Spiral
Ash value		
1	Total Ash	6.55% w/w
Moisture content		
1	Moisture content	15.60 %
Extractive values		
1	Alcohol soluble extractive value	11.65% w/w
2	Water soluble extractive value	21.63% w/w
3	Chloroform extractive value	4.35% w/w
4	Methanolicextractive value	15.58% w/w

Preliminary Phytochemical screening of extract

The Phytochemical analysis of Musa paradisica leaves showed the presence the presence of various secondary metabolites, including carbohydrates, alkaloids, flavonoids,saponins and terpenoids. These compounds play important roles in the biological activities and therapeutic potential of plant extracts.

Table 6. 2 Preliminary phytochemical test of extract

Sr. No.	Phytochemicals	Hydroethanolic extract
1	Alkaloids	+
2	Flavonoids	+
3	Carbohydrates	+
4	Saponin	+
5	Tannin	+
6	Glycosides	+
7	Anthraquinones	-
8	Steroids	+
9	Terpenoids	+

Note: Key:–absent, + present

Thin layer chromatography

The Rf value of extracts was evaluated using the following formula,

$$Rf\ value = \frac{Distance travelled\ by\ the\ solute\ (cm)}{Distance\ travelled\ by\ the\ solvent\ front (cm)}$$

The TLC profile of Musa paradisiaca leaf extract using the mobile phase Toluene: Ethyl Acetate: Ammonia (7:3:1) revealed distinct Rf values at 0.25, 0.42, and 0.71, indicating the separation of different compounds. When the TLC plate was observed under UV light at 365 nm, bright blue fluorescent spots appeared, suggesting the presence of basic nitrogenous compounds. Further confirmation was achieved by spraying the plate with Dragendorff's

reagent, which turned the spots orange-brown, a characteristic indication of alkaloids.

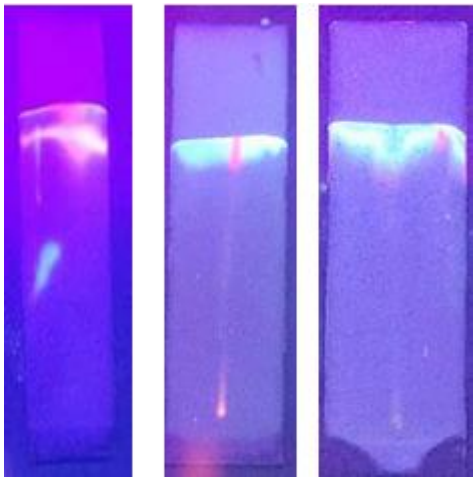

Figure : TLC profile of Musa paradisiaca leaf extract

UV Spectroscopy studies of plant extract

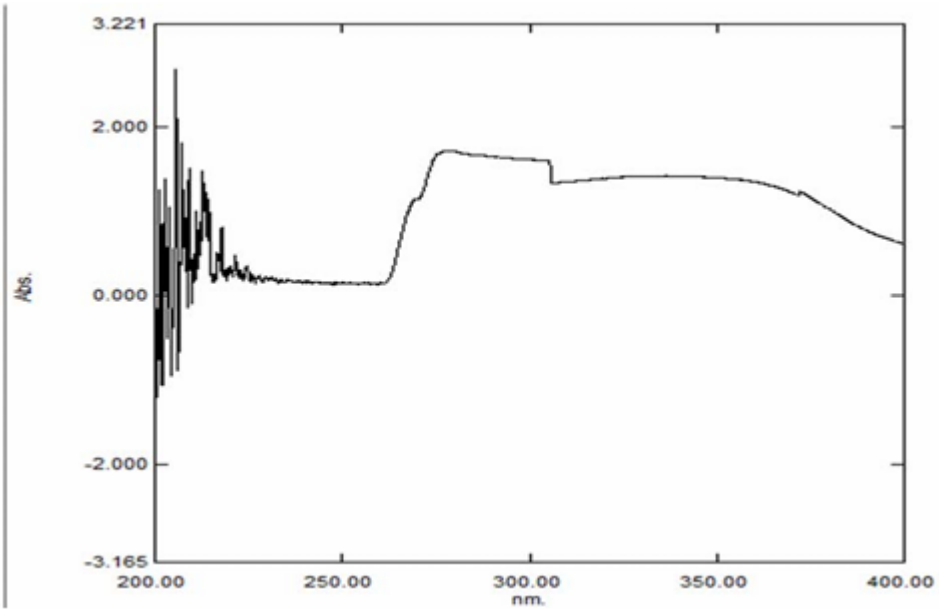

Figure : UV-Vis Spectrum Analysis of Musa paradisiaca Leaf Extract (Ethanol Fraction)

The UV-Visible spectroscopic analysis of the ethanolic fraction of Musa paradisiaca leaf extract exhibited a prominent absorbance peak at around 290 nm, indicating the presence of polyphenolic compounds, particularly flavonoids. A broad absorbance region extending up to 370 nm further supports the presence of conjugated phytochemical constituents. These results confirm that the extract contains bioactive compounds with potential antioxidant activity, which supports its traditional use in the treatment of various ailments, including hyperlipidaemia.

Acute Oral Toxicity of Musa paradisiac leaves extract

The initial investigation involved assessing the potential toxicity of hydroethanolic leaf extracts of Musa paradisica. A single rat was administered a relatively high dosage of 2000 mg/kg of extract and the results revealed no signs of toxicity. This indicates that the tested extracts did not cause adverse effects at the given concentration within the observed timeframe. Specifically, there were no reported deaths and no notable behavioural abnormalities were observed in any of the groups studied.

Pharmacological Evaluation of Extract

The oral administration of Musa paradisica leaf extract at doses of 200 mg/kg and 400 mg/kg resulted in a significant decrease in serum levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C). Conversely, there was a notable increase in serum high-density lipoprotein cholesterol (HDL-C) levels when compared to rats subjected to a high-fat diet (HFD). Results were tabulated in following table.

Table No.:The impact of leaf extract on cholesterol, triglyceride, and glucose levels in hyperlipidemia induced by a high-fat diet.

Groups	Cholesterol	Triglycerides	Glucose
Normal	95.05±1.833	76.73±1.147	75.58±0.995
Disease control	266.10±2.980***	184.35±2.810	136.43±1.564
HFD+Atrovastatin	115.81±1.984**	90.15±1.342**	92.98±1.210
HFD+Low Dose	238.06±2.362**	170.53±2.381***	119.95±1.495
HFD+HighDose	205.81±2.125**	134.3±1.896***	115.62±1.380

Table: Effect of test extract on high-density lipoprotein (HDL), low-density lipoprotein (LDL) &very low-density lipoprotein (VLDL) in hyperlipidemia induced by a high-fat diet.

Groups	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal	46.04±0.947**	32.06±1.822*	13.41±0.490
Disease control	25.13±0.894	206.02±3.986***	39.33±0.889
HFD+Atrovastatin	54.33±0.971**	40.25±2.033*	16.24±0.523**
HFD+Low Dose	30.42±0.862*	172.08±2.904*	33.30±0.675*
HFD+HighDose	40.76±0.960*	136.46±2.360***	25.37±0.662*

P values are indicated as follows: ***p<0.001, **p<0.01, and *p<0.05 in comparison to the normal group. The data are presented as mean ± standard error of the mean (SEM) from six animals in each group.

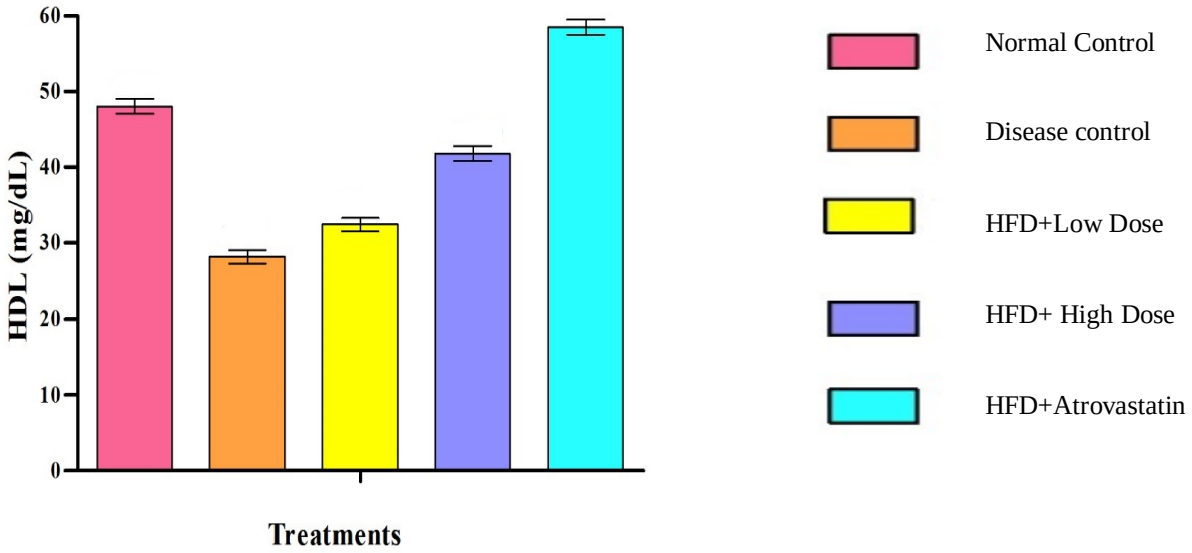

Figure : Effect of M. paradisiac extract on HDL level

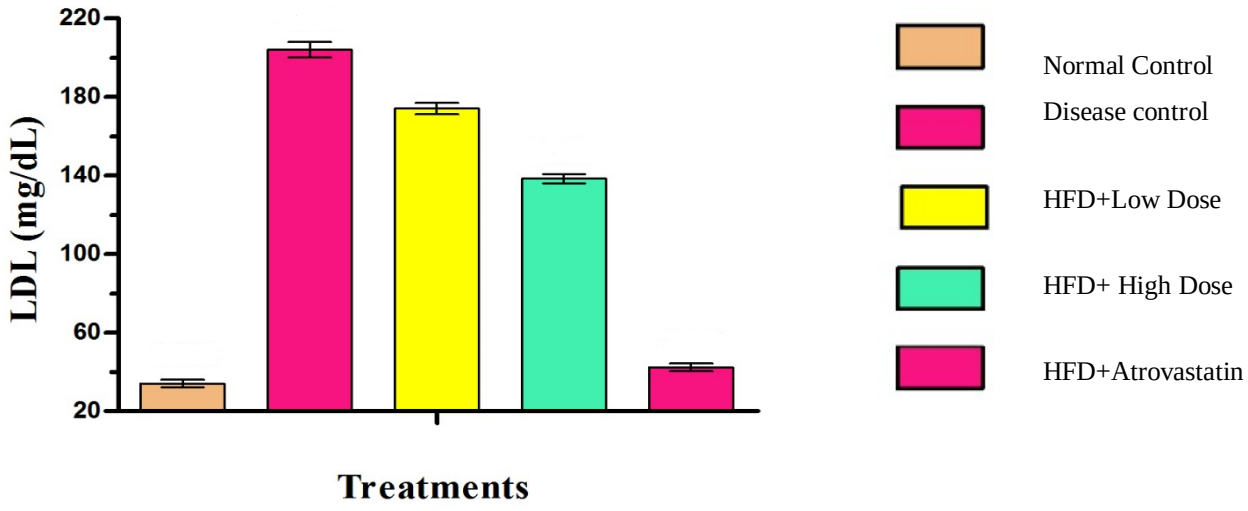

Figure : Effect of M. paradisica extract on LDL level

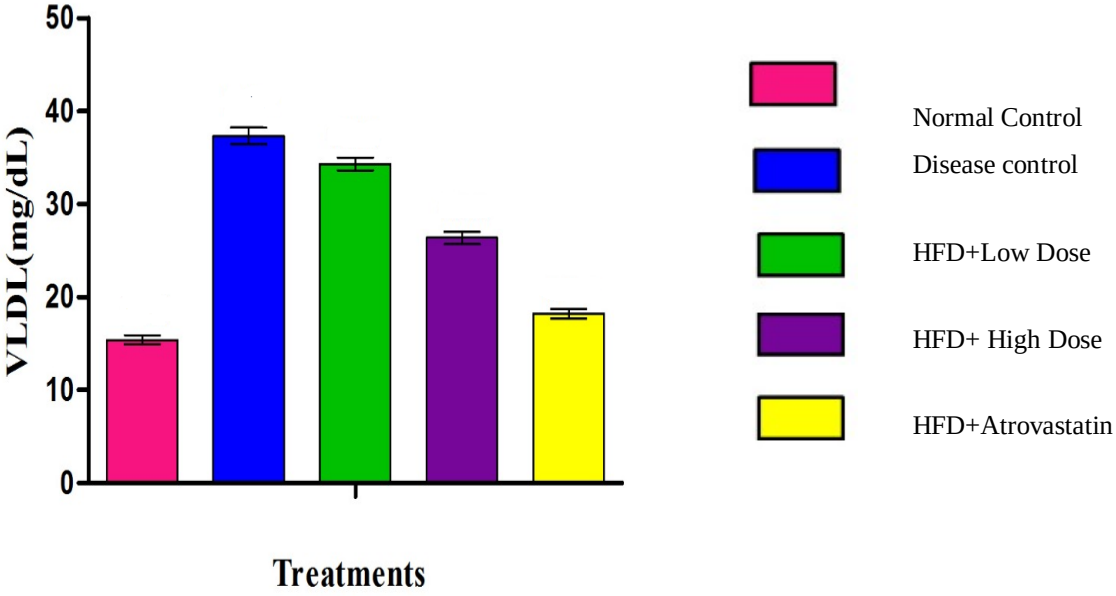

Figure : Effect of M. paradisica extract on VLDL level

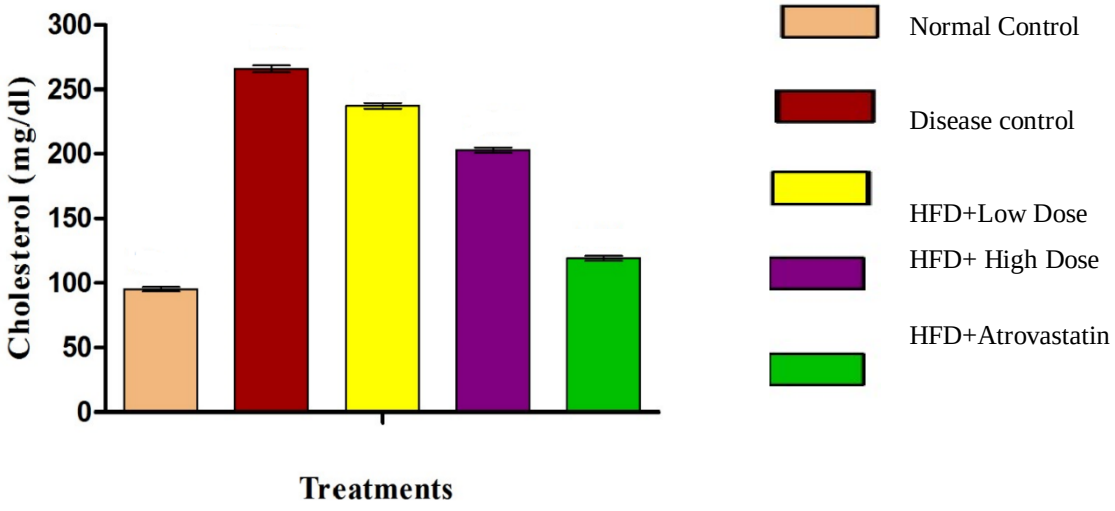

Figure : Effect of M. paradisica extract on Cholesterol level

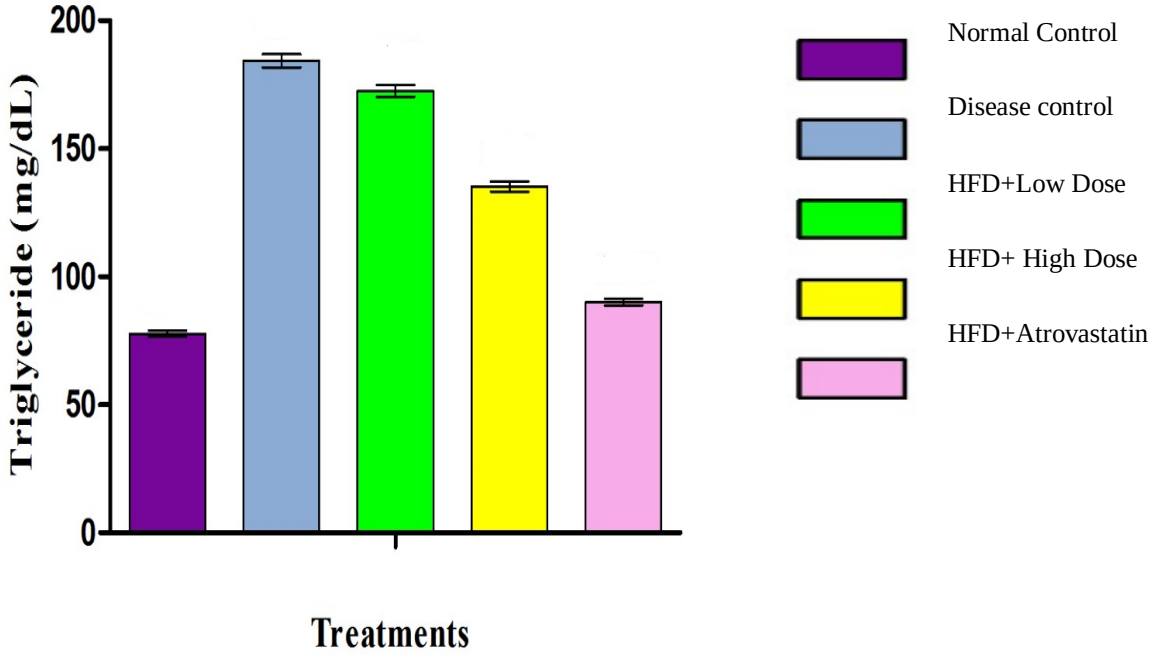

Figure : Effect of M. paradisica extract on Triglyceride level

Liver sinusoidal capillary dilations were observed under light microscopy in group II fed a high-fat diet, but not in group I fed a standard diet of chow. Hepatocytes of Group II animals fed a high-fat diet were fatty and vacuolated in their cytoplasm. Group V animals given atorvastatin had significantly fewer sinusoidal capillary dilatations and less cytoplasmic fat deposition and granular degeneration. Group III animals given 200 mg/kg had less sinusoidal capillary dilations, fat infiltration, and granular degeneration than group II animals fed a high-fat diet. Compared to animals fed a high-fat diet (group II), those given 400 mg/kg extract showed much less evidence of liver sinusoidal capillary dilations, cytoplasmic fatty infiltration, and granular degeneration.

(A) Hepatocytes in Group I (A) have a typical structure (Figure 6.22, I to V)

(B) Hepatocytes from Group II: Control (with HFD) show significant fatty infiltration into the cytoplasm and granular degeneration (B).

(C) Hepatocytes from the Group III: 200 mg/kg (High fat diet) animals show some fatty infiltration into the cytoplasm and granular degeneration.

(D) Microscopic examination of hepatocytes from Group IV: 400 mg/kg (on an HFD) revealed no cytoplasmic fatty infiltration and granular degeneration.

(E) Hepatocytes in the Group V: Standard atorvastatin medication 1.5 mg/kg (with HFD) show minimal fatty infiltration into the cytoplasm and granular degeneration.

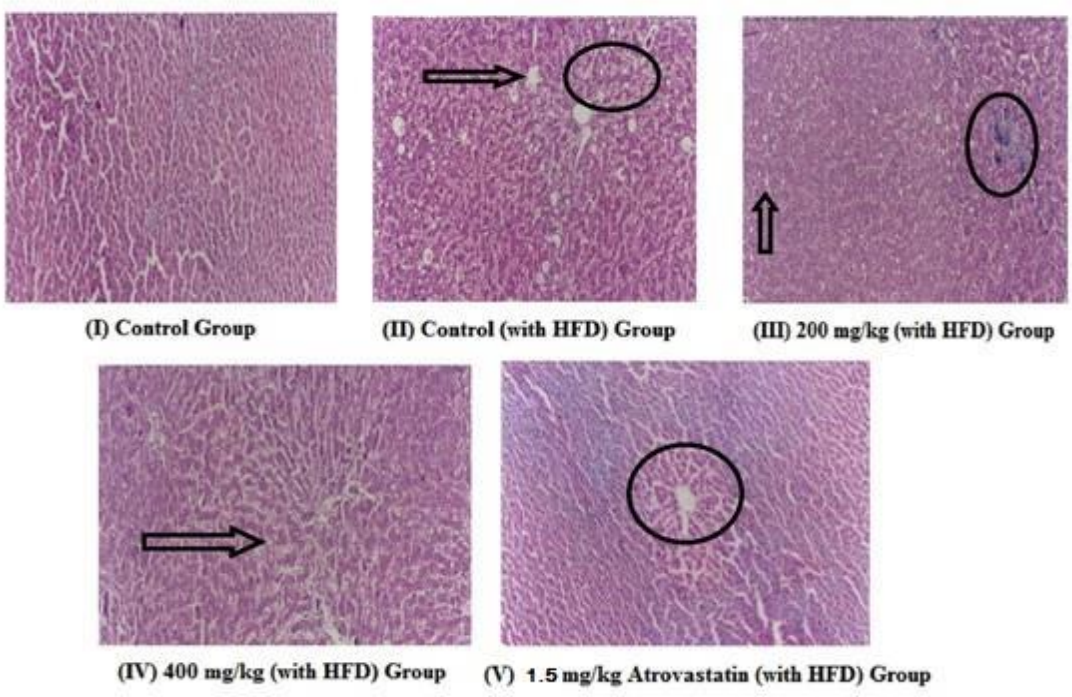

Figure: Histopathological analysis of liver tissue samples in the context of a high-fat diet Induced hyperlipidemic rat.

DISCUSSION:

Bioactive compounds found to be present in this plant include alkaloids, saponins, tannins, cardiac glycosides, terpenes, deoxy sugar, flavonoids and carbohydrates. The findings of this study is consistent with reports of the presence of these phytochemicals in various parts of the M. paradisiaca plant as documented by Akpuaka and Ezem (2011) and Akpabio, Udiong, and Akpakpan (2012). These phytochemicals have been reported to exert multiple biological and pharmacological effects (antibacterial, antihypertensive, antidiabetic and anti-inflammatory activities) (Ighodaro, 2012). The presence of these bioactive substances in M. paradisiaca leaves therefore suggests that the leaves possess valuable medicinal potential that can be explored.

In the acute toxicity assessment, no fatalities were observed throughout the treatment duration across all administered doses of the test extract. The subjects appeared to be in good health, exhibiting no signs of toxicity at doses up to 2000 mg/kg.

The oral administration of Musa paradisica leaf extract at doses of 200 mg/kg and 400 mg/kg resulted in a significant decrease in serum levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C). Conversely, there was a notable increase in serum high-density lipoprotein cholesterol (HDL-C) levels when compared to rats subjected to a high-fat diet (HFD).

The test extract remedies when taken appear to lower total cholesterol levels which the formulation was potent enough to lower its LDL component both in serum and in the liver in accordance with the targets of several hypolipidermic agents. From the results obtained it was showed that the lowering of cholesterol by this extract is possibly related to the increased breakdown of LDL cholesterol by liver receptors before its removal as bile acids.

Elevated serum levels of LDL-cholesterol are associated with a heightened risk of developing atherosclerosis. Conversely, it is widely recognized that HDL-cholesterol plays a protective role in coronary artery disease. Research indicates that HDL-cholesterol possesses a preventive effect against atherogenesis, as an independent inverse correlation has been observed between blood HDL-C levels and the incidence of cardiovascular risk. Furthermore, our extract has been shown to enhance HDL-cholesterol levels, thereby demonstrating anti-hyperlipidemic properties.

CONCLUSION:

The results of this study indicate that thehydroethanolic extract of Musa paradisica at the dose of 200mg/kg and 400mg/kg; p.o.showed good anti-hyperlipidemic action in High cholesterol diet induced hyperlipidemia model. The acute toxicity study indicated that the formulation are devoid of major toxic effects. The probable mechanism of action of the extract may be inhibition of HMG-CoA reductase enzyme pathway. As such, the hypolipidaemic activity of M. paradisiaca can be exploited for the treatment of hyperlipidemia caused by the intake of high fat diet at relatively low doses to avoid toxicity.

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