

**ANTI-OBESITY AND ANTI-HYPERLIPIDEMIC ACTIVITY OF MURRAYA
PANICULATA LINN. LEAF HYDROETHANOLIC EXTRACTS IN CAFETERIA
DIET INDUCED OBESE RATS**

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**INTRODUCTION
HYPERLIPIDEMIA**

Hyperlipidemia is abnormally elevated levels of any or all lipids or lipoproteins in the blood. Hyperlipidemia is an umbrella term that refers to acquired or genetic disorders that result in high levels of lipids (fats, cholesterol, or triglycerides) circulating in the blood. This disease is usually chronic and requires ongoing medication to control blood lipid levels.

Lipids (water-insoluble molecules) are transported in a protein capsule. The size of that capsule, or lipoprotein, determines its density. The lipoprotein density and type of apolipoproteins it contains determines the fate of the particle and its influence on metabolism. Hyperlipidemias are divided into primary and secondary subtypes. Primary hyperlipidemia is usually due to genetic causes (such as a mutation in a receptor protein), while secondary hyperlipidemia arises due to other underlying causes such as diabetes. Lipid and lipoprotein abnormalities are common in the general population and are regarded as modifiable risk factors for cardiovascular disease due to their influence on atherosclerosis. In addition, some forms may predispose to acute pancreatitis.

ANTIHYPERLIPIDEMIC DRUGS

Antihyperlipidemic agents promote reduction of lipid levels in the blood. Some antihyperlipidemic agents aim to lower the levels of low-density lipoprotein (LDL) cholesterol, some reduce triglyceride levels, and some help raise the high-density lipoprotein (HDL) cholesterol. Hypolipidemic agents, cholesterol-lowering drugs or antihyperlipidemic agents, are a diverse group of pharmaceuticals that are used in the treatment of high levels of fats, such as cholesterol, in the blood. They are called lipid-lowering drugs.

Hyperlipidemia characterized by hypercholesterolemia is the most prevalent indicator for susceptibility to cardiovascular diseases. World health organization reports that high blood cholesterol contributes to approximately 56% of cases of cardiovascular diseases worldwide and causes about 4.4 million deaths each year. Hyperlipidemia is a metabolic disorder, specially characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentrations of Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), and triglycerides (TAG) with a concomitant decrease in the concentrations of high density lipoprotein cholesterol (HDL-C) in the blood circulation. Currently, the use of alternative medicines and especially the consumption of phytochemicals have been rapidly increasing worldwide. As herbal medicines

are less damaging than synthetic drugs they have better compatibility thus improving patient tolerance even on long-term use.

OBESITY

Obesity is a complex interplay between environmental and genetic factors and is associated with significant morbidity and mortality. Obesity is difficult to define in quantitative term. Obesity refers to the above average amount of fat contained in the body, this in turn is dependent on the lipid content of each fat cell and on the total number of fat cells. By 2005, obesity had affected 400 million adults, and since 1997, WHO has cited obesity as a global epidemic. More than half of the adult population in OECD countries is overweight (body mass index [BMI] ≥ 25 kg/m²). The obesity incidence has increased at an alarming rate in recent years, becoming a worldwide health problem, with incalculable social costs. According to WHO, obesity is related to cardiovascular diseases, hypertension, diabetes mellitus, cancer, osteoarthritis, pulmonary diseases, as well as psychological issues, including social bias, prejudice, discrimination, and overeating. Economically, obesity and its health consequences contribute to enormous costs now and for future health care. Therefore, prevention and treatment of obesity are important for a healthy life. However, owing to the adverse side effects associated with many synthetic anti-obesity drugs, more recent trials have focused on screening natural sources that have

been reported to reduce body weight with minimal side effects. This may be an excellent alternative strategy for developing effective and safe anti-obesity drugs in the future. Single and mixed anti-obesity medicinal plant preparations may have different effects. The botanical sources, route of administration, presence of various bioactive components and their respective functions, experimental methods used, treatment dosage, study design, treatment duration, and safety and efficacy of the plant are also factors.

Obesity is a medical condition in which excess body fat has accumulated to an extent that it may have a negative effect on health. People are generally considered obese when their body mass index (BMI), a measurement obtained by dividing a person's weight by the square of the person's height despite known allometric inaccuracies is over 30 kg/m^2 ; the range $25\text{--}30 \text{ kg/m}^2$ is defined as overweight. Some East Asian countries use lower values. Obesity is correlated with various diseases and conditions, particularly cardiovascular diseases, type 2 diabetes, obstructive sleep apnea, certain types of cancer, and osteoarthritis. High BMI is a marker of risk, but not proven to be a direct cause, for diseases caused by diet, physical activity, and environmental factors. A reciprocal link has been found between obesity and depression, with obesity increasing the risk of clinical depression and also depression leading to a higher chance of developing obesity.

Effects on health

Excessive body weight is associated with various diseases and conditions, particularly cardiovascular diseases, diabetes mellitus type 2, obstructive sleep apnea, certain types of cancer, osteoarthritis, and asthma. As a result, obesity has been found to reduce life expectancy.

HIGH FAT DIET

The diet-induced obesity model (DIO model) is an animal model used to study obesity using animals that have obesity caused by being fed high-fat or high-density diets. It is intended to mimic the most common cause of obesity in humans. Typically mice, rats, dogs, or non-human primates are used in these models. These animals can then be used to study in vivo obesity, obesity's comorbidities, and other related diseases. Users of such models must take into account the duration and type of diet (e.g. hydrated gels vs. dry pellets) as well as the environmental conditions and age of the animals, as each may promote different bodyweights, fat percentages, or behaviors. Driven by the worldwide epidemic of obesity, particularly in the Western world, the DIO model has been integral in understanding the relationship between high-fat/high-density diets and obesity, including the discovery of Akt and mTOR, signals in the body linked to obesity and insulin resistance.

MATERIALS AND METHODS

Materials

Test Drug- *Murraya Paniculata*

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

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

- (Proposal no RCOP/IAEC/AUG/2021/05)

Housing Conditions

In separate cages 12-12hour light and dark cycle

- **Relative Humidity -** 40-60%
- **Temperature -** 25°C ($\pm 2^\circ\text{C}$)
- **Diet-** Standard Food pellets consumed by Rats

Instrument

Soxhlet apparatus (KHERA).

Method

Preliminary work (Selection of Plant)

Gathering sufficient information from various articles and journals it was concluded that there is scope to explore some more pharmacological activities in the plant *Murraya paniculata* hence it was selected for further studies.

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 with **Voucher specimen no (To be allocated)**

Drying, size reduction and storage of plant material

- 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.
- Packing to maintain uniformity and packed into airtight container and stored in cool and dry place. This material was used for the further study.

Preparation of *Murraya Paniculata* leaves (L.) extract

Extraction of *Murraya paniculata* was done by Soxhlet extraction method. (using standard method).

The process of extraction is depicted in figure

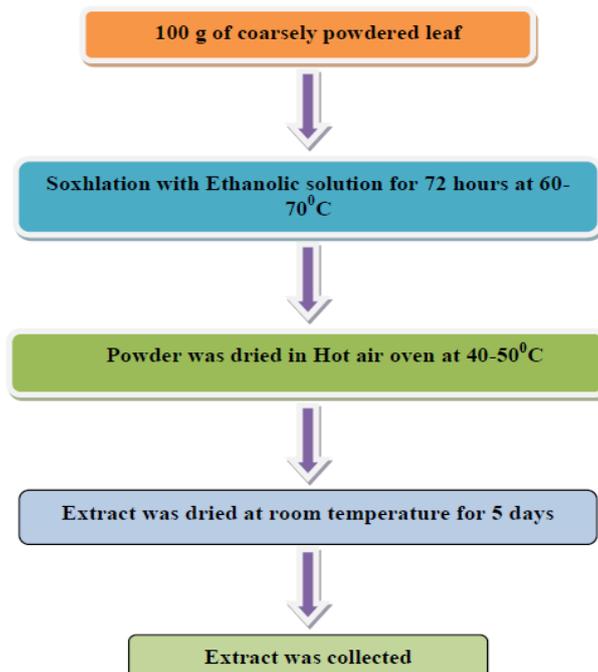


Figure no. 01: Extraction Process (Soxhlet apparatus).

Phytochemical Analysis Of Crude Extracts (Khandwelkre1997)

The crude extracts of plants obtained by solvent extraction were subjected to various qualitative tests to detect the presence of common chemical constituents as: alkaloid, glycoside, carbohydrate, phytosterols, saponins, tannin, flavonoids and protein etc.

Test for alkaloids

- **Dragendroff's test:** In the test tube 1 ml of extract and 1 ml of Dragendroff's reagent (potassium bismuth iodide solution) was added. An orange-red precipitate was appeared which indicated the presence of alkaloids.
- **Mayer's test:** In the test tube 1 ml of extract and 1 ml of Mayer's reagent (Potassium mercuric iodide solution) were added. Whitish yellow or cream colored precipitate indicated the presence of alkaloids.
- **Wagner's test:** In the test tube 1 ml of extract and 1 ml of Wagner's reagent (Iodine potassium iodide solution) were added. Reddish brown precipitate indicated the presence of alkaloids.

Test for carbohydrates, gums and mucilage

- **Benedict's test:** To 5ml of Benedict's reagent, 1ml of extract solution was added and boiled for 2 minutes and cooled. Formation of red precipitate indicated the presence of sugars.
- **Molisch's test:** A small fraction of extract was taken in ethanol separately and a few drops of 20% w/v solution of α -naphthol in ethanol (90%) were added to it. After shaking well, about 1 ml of concentrated sulphuric acid was allowed to flow carefully by the

side of the test tube. A reddish violet ring at the junction of the two layers indicated the presence of carbohydrates.

- **Fehling's test:** Extract heated with dil. HCL than neutralized with NaOH than added Fehling's solution A & B. Brick red precipitate was formed. It's indicated the presence of carbohydrates.
- **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 0.08 gm of ruthenium red dissolved in 10 ml of 10% solution of lead acetate, it stains the mucilage to red color.

Tests for glycosides

- **Killer-killani test:** Dissolved 2 ml of extract in Glacial acetic acid and then added one drop of 5% FeCl_3 and conc. H_2SO_4 . Reddish brown color appeared at the junction of the two liquid layers and upper appeared bluish green indicated the presence of glycosides.
- **Baljet's test:** To 1ml of the test extract, 1ml of sodium picrate solution was added and the yellow to orange color revealed the presence of glycoside.
- **Foam test:** 0.5gm extract vigorously shaken with water than formation of a layer of foam. It's indicated the presence of glycosides.

Test for flavonoids

- **Shinoda test:** To the test solution add few magnesium turnings and concentrated hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

Test for phenolic compounds and tannis

- **Test with lead acetate:** Tannins get precipitate with lead acetate.
- **Test with ferric chloride:** Generally phenols were precipitated with 5% w/v solution of ferric chloride in 90% alcohol thus phenols are detected.
- **Test with gelatin solution:** To a solution of tannins (0.5 – 1%) aqueous solution of gelatin (1%) and sodium chloride (10%) were added. A white buff precipitate confirms the compounds.

Test for Proteins

- **Biuret test:** Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO₄ solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet color indicates the presence of protein.

Test for Saponin

- **Froth test:** A little fraction of extract was boiled with about 1 ml of distilled water and shaken. Appearance of a characteristic foam formation indicated the presence of Saponins. Aqueous and alcoholic extract were tested directly.
- **Foam test:** A little fraction of extract was taken with about 2 ml of distilled water. A small quantity of sodium carbonate was added to each and shaken. The characteristic foam formation indicated the presence of Saponins. Aqueous and alcoholic extract were tested directly.

Test for Steroids

- **Salkowski test:** The extract was dissolved in chloroform and equal volume of conc. H₂SO₄ was added. Formation of bluish red to cherry color in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.
- **Liebermann-Burchard test:** A small portion from extract was taken with about 1 ml of acetic anhydride and dissolved by warming. The contents were cooled and a few drops of concentrated sulphuric acid were added in each case by the sides of the test tube. Appearance of blue color indicated the presence of sterols.

Experimental Work

Animals: Adult Wistar rats of 180-250 g were used for the study. The rats were obtained from the Ravishankar college of pharmacy Bhopal, (M.P.) for experimental purpose. The animals were maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%) and 12 h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for 48 h prior to experimental

protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Ravishankar college of pharmacy Bhopal, (M.P.) According to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animal, Govt. of India. (Approval no. 1733/PO/Ere/S/13/CPCSEA).

Preparation of dose

The test extract *Murraya paniculata leaf* part of dissolved in suspending agent (1% CMC) before orally administered to the Rats.

Standard (Atorvastatin) drug --- dissolved in suspending agent (1% CMC) before orally administered to the Rats.

Toxicity Study

The acute oral toxicity test of methanolic leaf powder extract was determined prior to the efficacy study as per the OECD (Organization for Economic Cooperation and Development) 425 Guidelines. Female Albino Swiss mice were administered the *Murraya Paniculata Leaf powder* (TCLP) assessing low dose of 2000 mg/kg body weight. The treated animals were observed for 14 days for mortality, clinical signs and symptoms. There was no mortality even at higher dose of 2000 mg/kg body weight.

Experimental Protocol

Animals were divided into five groups with of six animals in each group. The animals were identified by picric acid marking. The experimental design was as follows.

- Group 1: Normal control treated with saline only (Saline water)
- Group 2: Positive control treated with Cafeteria Diet (CD)
- Group 3: Treated with Standard drug (Atorvastatin) (10 mg/kg body weight) + CD
- Group 4: Treated with ethanolic extract of *Murraya paniculata Leaf* (250mg) + CD
- Group 5: Treated with ethanolic extract of *Murraya paniculata Leaf* (350mg) + CD

The food intake of each animal was determined initially and then every week thereafter by measuring the difference between the preweighed chows and the weight of the food that remained after 24 hours, and the results were expressed as a mean energy intake for a group of six rats in kcal/week.

On day 42, blood was collected by retro-orbital puncture from the ether-anesthetized rats and subjected to centrifugation to obtain the serum. The serum levels of glucose, total-cholesterol, high density protein (HDL), LDL, and triglycerides (TGs) were estimated using the biochemical kits.

Composition of Cafeteria diet

Ingredients	Calorie value(kcal/100 g)
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Condensed milk	335
Bread	230
Chocolate	550
Biscuit	360
Dried coconut	660
Cheese	320
Boiled potato	80

RESULTS

Morphology

Table: Morphological characteristics of leaves of *Murraya paniculata* (L).

S. No.	Character	Observation
1	Color	Green
2	Odor	Pungent
3	Taste	Characteristic
4	Size	20-50 cm. length

Consistency and color

Table: Consistency and color of leaves of *Murraya paniculata* (L).

Extract	Color	Consistency
Hydro alcoholic	Greenish	Semi solid

Practical & Percentage Yield

Table: Percentage yield of leaves of *Murraya paniculata* (L) hydroethanolic extract.

S. No	Extract	Yield (gm)	Percentage Yield
1	Hydro alcoholic	10.50	9.25 %

Phytochemical screening: There is a presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins and saponins in hydro-alcoholic extract of *Murraya paniculata* (L)

Table: Phytochemical screening of hydro-alcoholic extract of *Murraya paniculata* (L).

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	Liebermann-Burchard test	+
		Salkowski test	+
7	Saponins	Foam test	+
8	Protein	Xanthoprotic	+
9	Gums & Mucilage	With 95% alcohol	-

(+) = Present, (-) = Absent

Evaluation Parameters

All values are Mean \pm SEM, n=06, *P<0.05, **P<0.01, when compared to vehicle treated group as well as control group (Following repeated measures ANOVA, Parametric methods, using Dunnett Test)

Effect on Delonix Regia extracts on Serum Biochemical Parameters

Groups (06 animals / Group)	Dose mg/kg	Diet	HDL-Cholesterol mg/dl	Body Weight (Change in %)
Standard Group	Atorvastatin 5 mg/ kg/ day p.o.	HFD + NFD (1:1) for 40 days	46.60 \pm 0.2 ^{oo}	21.22%
Test Group-I (HEMP)	Plant extract 250 mg/ kg / day p.o.	(6 weeks approx / 40 days study model)	56 \pm 0.02 ^o	11.44%
Test Group-II (HEMP)	Plant extract 350 mg/ kg/ day p.o.		33.605 \pm 7.303 ^o	9.26 %
Normal Control	Normal Fed Diet (pellets)		33.28 \pm 0.01 ^o	-

DISCUSSION

Various animal models of obesity have been used to evaluate obesity like condition in humans, in order to

develop effective antiobesity treatments. Among the animal models of obesity, rats that are fed a high fat diet are considered useful; a high percentage of fat in their

diet is considered to be an important factor in the development of obesity, leading to the accumulation of body fat, even in the absence of an increase in calorie intake. The present study showed that the administration of a CD for six weeks, in rats, produced obesity like conditions, with increase in body weight and serum lipid levels. Furthermore, it also induced a fatty liver with the accumulation of hepatic triglycerides. Treatment with DRE at a dose of 350 mg/kg/day significantly reduced the increase in body weight induced by a CD — a clear sign of an antiobesity effect. Also dose was found to be potent as compared to 250 mg/kg/day. This result suggests that the body weight reducing effect of DRE in cafeteria dietfed rats may be produced due to its hypophagic property. The extract produced a significant decrease in the total body weight in comparison with the CD control group.

CONCLUSION

The chemical components from different parts of *M. paniculata* were identified using chromatographic techniques and the structures were elucidated using spectroscopic techniques. A number of these compounds exhibited significant biological activities, which serve as the scientific evidence for the traditional usage of *M. paniculata*. Phytoconstituents such as alkaloid, called Yuehcukene, 1β -(3-indolyl)-7,9 α ,9 β -trimethyl-5 β ,8,9,10 β -tetrahydroindano-[2,3-b] indole was also isolated from *M. paniculata* leaves. Moreover, the two indole alkaloids, *Murraya carine* and *murraya culatine* were isolated from root bark and flowers of *m. paniculata* respectively. Also other compounds such as identified from volatile and essential oil extracted from *M. paniculata* leaves. The major components were γ -caryophyllene, γ -elemene, perolidol, β -elemene, spathulenol, caryophyllene oxide, β -caryophyllene, germacrene D and 4-methylene-6-(1-propenylidene)-cyclooctene. On basis of literature review obtained it is clearly demonstrate that *M. paniculata* possess the high potential for in vitro and in vivo studies. The current state of research on *M. paniculata* implicates great potential of the isolated bioactive compounds in treating diseases. Therefore, the chemical study of extract *M. paniculata* leaves and its pharmacological uses reported earlier with review of literature, provide the base for Antihyperlipidemic activity using High fat diet Induced Model in experimental rats.

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