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# ANTI-HYPERLIPIDEMIC POTENTIAL OF METHANOL EXTRACT OF *STACHYTARPHETAURTICIFOLIA* IN HIGH-FAT DIET-INDUCED OBESE RATS: A COMPREHENSIVE EVALUATION

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**ABSTRACT**

Hyperlipidemia is characterized by an elevated level of lipids, including cholesterol and triglycerides, in the plasma, which increases the risk for cardiovascular diseases. It involves the disruption of lipid homeostasis, where an imbalance between different lipoproteins—chylomicrons, VLDL, LDL, and HDL—can lead to pathological conditions. Cholesterol, both exogenous and endogenous, plays a critical role in cellular functions, such as membrane fluidity, hormone synthesis, and bile salt production. Triglycerides, the body's main energy reserves, are primarily stored in adipose tissue and serve as an energy source when needed. The absorption of dietary lipids occurs in the small intestine, where pancreatic lipase hydrolyzes dietary fats, and chylomicrons are formed for fat transport. Lipoproteins, such as VLDL and LDL, transport triglycerides and cholesterol to tissues, while HDL functions in reverse cholesterol transport, returning excess cholesterol to the liver for metabolism. Understanding the complex interplay between lipoproteins and lipid metabolism is crucial for developing therapeutic strategies for hyperlipidemia, such as statins and other lipid-lowering agents.

**Keywords**: Diabetic Hyperlipidemia, Lipoproteins, Cholesterol, Triglycerides, Lipid metabolism.

## INTRODUCTION

### Hyperlipidemia

Hyperlipidemia is an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids and or plasma lipoprotein including very low-density lipoprotein and low-density lipoprotein, and reduced high-density lipoprotein levels.[1]

### Intestinal Lipid Absorption

Growing bodies of evidences indicate, both in humans and animal models, that the small intestine is not only involved in the absorption of dietary lipids but actively regulates the production and secretion of CMs.

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The process of dietary lipid absorption is traditionally divided into three components: (a) uptake into the erythrocyte, (b) intracellular processing, and (c) transport into the circulation. [2] Pancreatic lipase makes the first step possible through the hydrolysis of dietary fats, mostly triacylglycerols (TAG), within the lumen of the small intestine. Fatty acids (FA) and sn-2-monoacylglycerol (MAG) are the results of this enzymatic breakdown.[3] Hydrolysis products are then transported across the apical brush border membrane of the enterocyte by cluster determinant 36 (CD 36). [4] The FA are then bound by FA binding proteins (FABPs) and targeted to microsomal compartments for re-esterification to triglycerides. De-novo lipogenesis represents another valid source of triglycerides useful for lipidation and this process is hormone-dependent.[2] CM assembly is a complex process that needs the activity of microsomal triglyceride transfer protein (MTP) to cotranslationally

incorporate apoB-48 into a phospholipids-rich, dense, primordial chylomicron particle (prechylomicron).[5] Then, prechylomicrons are included in a unique transport vesicle, the prechylomicron transport vesicle (PCTV), which is budded off the endoplasmic reticulum (ER) membrane and transported to the Golgi. Once into the Golgi compartment several chylomicrons fuse into another transport vesicle and are transported to the basolateral membrane for secretion in the circulation. Two different models have been proposed for CMs assembly. According to Hussain, the assembly of small nascent lipid poor CM particles and buoyant triglyceride-rich chylomicrons progress through independent pathways.[6] On the other hand the so called "core expansion" model, proposes that primordial chylomicrons and triglyceride-rich lipid droplets of various sizes join together to form lipoproteins of different size [7].

**Cholesterol**

Cholesterol is a waxy fat molecule that the liver produces.[8] It is a major sterol in animal tissues, has a significant function in the human body. Cholesterol is a structural component of cell membranes and plays an integral role in membrane fluidity. Cholesterol is also important in the synthesis of lipid rafts which are needed for protein sorting, cellular signaling, and apoptosis. [9] Cholesterol is derived both from the diet and by endogenous synthesis in the liver and it is a component of all cell membranes, a precursor of steroid hormones including estrogen, progesterone, testosterone, as well as vitamin D and bile salts, and of glycoproteins and quinones. The biochemistry and metabolism of cholesterol is complex. Cholesterol and other lipid fractions are transported in blood via lipoproteins of different densities. [10, 11]

**Triglycerides**

Triacylglycerols (also called as triglycerides) are the most abundant lipids comprising 85-90% of body lipids. Most of the triglycerides (TG; also called neutral fat or depot fat) are stored in the adipose tissue and serve as energy reserve of the body. This is in contrast to carbohydrates and proteins which cannot be stored to a significant extent for energy purposes. Fat also acts as an insulating material for maintaining the body temperature of animals. [12] Triglycerides are the most predominant storage form of energy. There are two main reasons for fat being the fuel reserve of the body.

**Lipoproteins**

Lipoproteins are macro molecules aggregate composed of lipids and proteins; this structure facilitates lipids compatibility with the aqueous body fluids.[4] While in circulation, cholesterol, being a lipid, requires a transport vesicle to shield it from the aqueous nature of plasma. Complex, micelle-like amalgamations of various proteins and lipids achieve cholesterol transport through the vascular system. These particles, intuitively known as lipoproteins, are heterogeneous in size, shape, composition, function.[14] Lipoproteins deliver the lipid components (cholesterol, triglycerides etc.) to various tissues for utilization.[15] Homeostasis of cholesterol is centered on the metabolism of lipoproteins, which mediate transport of the lipid to and from tissues.[14] Plasma lipoproteins are separated by hydrated density; electrophretic mobility; size; and their relative content of cholesterol, triglycerides, and protein into five major classes: chylomicrons, very-low-density lipoproteins (VLDL), intermediate-density lipoprotein (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).[16]

**Chylomicrons**

A small fat globule composed of protein and lipid. The chylomicrons are synthesized in the mucosa (the lining) of the intestine and are found in the blood and lymphatic fluid where they serve to transport fat from its port of entry in the intestine to the liver and to adipose tissue. After a fatty meal, the blood is so full of chylomicrons that it looks milky.[17]

**Very Low-Density Lipoproteins (VLDLs)**

VLDLs are produced by the liver and are triglyceride rich. They contain apolipoprotein B-100, C-I, C-II, C-III, and E. APO B-100 is the core structural protein and each VLDL particle contains one APO B-100 molecule. Similar to chylomicrons the size of the VLDL particles can vary depending on the quantity of triglyceride carried in the particle,[18] but their triglyceride content is lower and cholesterol content higher than that of chylomicrons. Like chylomicrons, VLDLs are substrates for lipoprotein lipase-mediatedtriglyceride removal. Their function is to carry triglycerides synthesized in the liver and intestines to capillary beds in adipose tissue and muscle, where they are hydrolyzed to provide fatty acids that can be oxidized to produce adenosine triphosphate (ATP) for energy production. Alternatively, if not needed for energy production, they can be re-esterified to glycerol and stored as fat. After removal of their triglyceride, VLDL remnants (called IDLs) can be further metabolized to LDL. VLDLs serve as acceptors of cholesterol transferred from HDL. This transfer process is mediated by an enzyme called cholesterol ester transfer protein (CETP).[19]

**Intermediate Density Lipoprotein (IDL)**

Intermediate density lipoproteins (IDL) are also called as the VLDL remnants. These lipoprotein s are less dense than LDL molecules but denser than VLDL particles. As the triglycerides on VLDL are broken down

by the cells that need it, the particle becomes denser due to the change in the lipid to protein ratio. This results in VLDL being converted into IDL. Each native IDL particle consists of protein that encircles various fatty acids, enabling, as a water-soluble particle, these fatty acids to travel in the aqueous blood environment as part of the fat transport system within the body. IDL enable fats and cholesterol to move within the water-based solution of the bloodstream. Their size is, in general, 25 to 35 nm in diameter, and they contain primarily a range of triacylglycerols and cholesterol esters. They are cleared from the plasma into the liver by receptor-mediated endocytosis, or further degraded to form LDL particles. [20, 21, 22]

**Low Density Lipoprotein (LDL)**

These particles are derived from VLDL and IDL particles and they are even further enriched in cholesterol. LDL carries the majority of the cholesterol that is in the circulation. The predominant apolipoprotein is B-100 and each LDL particle contains one APO B-100 molecule. LDL consists of a spectrum of particles varying in size and density. An abundance of small dense LDL particles are seen in association with hypertriglyceridemia, low HDL levels, obesity, type 2 diabetes (i.e. patients with the metabolic syndrome) and infectious and inflammatory states. These small dense LDL particles are considered to be moreproatherogenic than large LDL particles for a number of reasons. Small dense LDL particles have a decreased affinity for the LDL receptor resulting in a prolonged retention time in the circulation. Additionally, they more easily enter the arterial wall and bind more avidly to intra-arterial proteoglycans, which traps them in the arterial wall. Finally, small dense LDL particles are more susceptible to oxidation, which could result in an enhanced uptake by macrophages.[18]

**High Density Lipoprotein (HDL)**

HDLs are heterogeneous particles regarding their size and composition. Compared with other lipoproteins, they have the highest relative density while being smallest in size. HDL have an important role in carrier in reverse cholesterol transport (RCT) and act as a carrier of cholesterol back to the liver. They effectively function in homeostasis and lipid metabolism. HDL is mainly secreted by the liver and small intestines. The liver, which secretes ~70-80% of the total HDL in plasma, is the main source of HDL in the circulation. Apolipoprotein(apo)AI is the major structural protein and constitutes the framework of HDL to bear phospholipids and cholesterol. In addition to apoAI, several other apolipoproteins (for example, apoAII, apoAIV, apoB, apoCI and apoCII) contribute to the composition of HDL. HDL particles are highly uniform and can be divided into several sub-types based on their composition proteins or bulk density.[23]

**SCOPE & PLAN OF WORK**

Scientists have become increasingly interested in studying free radicals and trying to discover new antioxidant phytochemicals over the past ten years due to their wide-ranging effects on biological systems. The link between free radicals and the onset of chronic and acute illnesses, including cancer, cardiovascular disease, rheumatoid arthritis, inflammation, cataracts, diabetes, Alzheimer's disease, and ageing, is well established. Reactive oxygen or nitrogen species damage results in pathological alterations that are linked to a variety of illnesses. Stachytarphetaurticifolia is a traditional medicinal plant that is used all over the world for its medicinal properties. Studies have shown its efficacy as a diuretic, tonic, anti-syphilitic, and vermifuge, as well as an anthelmintic and treatment for skin conditions. As a result, the antihyperlipidemic efficacy of Stachytarphetaurticifolia methanol and aqueous extracts was assessed.

**Morphology**

It is frequently named as blue snakeweed and nettle leaf velvet berry. It is a perennial herb growing wild in the Sylhet and Chittagong districts of Bangladesh and also cultivated as an ornamental weed. Species present in Africa, in the islands of the Indian Ocean (Seychelles, Comoros, Madagascar, Reunion, Mauritius), in South-East Asia, in the east of Australia, in New Caledonia and in the Pacific islands.

**Distribution and habitat**

It is 0.5–1.5 m tall with ovate to elliptic ovate, or oblong, serrate leaves and 4 angled softly pubescent stems. Calyx lobes are shortly 5 toothed and corolla dark purple-blue, mauve or royal blue with a light or white throat. The leaves are opposite and simple. The petiole, 0.5 to 2 cm long, is winged by the extension of the lamina. The lamina is oval or elliptical, 3 to 8 cm long and 2 to 4.5 cm wide, base attenuated on the petiole, with acute apex, margin cut into pointed triangular tines. Its surface, corrugated on the upper side, is smooth except for a weak pubescence on the ribs and the base of the young leaf, on the underside.

**Pharmacological Activities**

**Antibacterial activity**

The aqueous extract of S. indica root and methanolic extract of Stachytarphetaurticifolia leaf showed significant activity against various species of bacteria which is comparable with standard antibiotic streptomycin and ampicillin, respectively. Several studies have demonstrated the antimicrobial potential of S.

jamaicensis extracts toward pathogenic microorganisms, including bacteria and fungi.

## MATERIALS AND METHODS

### Collection of Plant Material

Stachytarphetaurticifolia. leaves were procured from Thalakona locality in Tirupathi. Leaves of Stachytarphetaurticifolia were shade dried under room temperature for one week and leaves were powdered mechanically. The finely powdered leaves were kept separately in an air tight container until the time of use. About 50 gms of finely powdered leaves were extracted using Methanol in Soxhlet apparatus and on the other hand maceration was performed with same amount of drug powder under sonication for 24 hours and finally solvents were evaporated and concentrated by using rotary evaporator. These leaf extracts were used for phytochemical screening anti-hyperlipidemic activities

### Phytochemical Analysis

The concentrated extracts were subjected to chemical tests as per the methods mentioned below for the identification of the various constituents as per the standard procedures given by Kokate and Trease and Evans.

(1) Detection of Alkaloids

Small portions of solvent-free chloroform, alcohol and aqueous extracts were stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal reagents.

- Mayer's test: Filtrates were treated with potassium mercuric iodide (Mayer's reagent) and the formation of cream coloured precipitate was indicates the presence of alkaloids.
- Dragendroff's test: Filtrates were treated with potassium bismuth iodide (Dragendroff's reagent) and formation of reddish brown precipitate was indicates the presence of alkaloids.
- Wagner's test: Filtrates were treated with solution of iodine in potassium iodide (Wagner's reagent) and formation of brown precipitate was indicates the presence of alkaloids.
- Hager's test: Filtrates were treated with a saturated solution of picric acid (Hager's reagent) and formation of yellow precipitate was indicates the presence of alkaloids.

### In-vivo Pharmacological Screening

**Definition:**

Toxicity is the degree to which a substance is able to damage an exposed organism. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicty) or an organ (organotoxicity), such as the liver (hepatotoxicity).

**Types of toxicity**

There are generally three types of toxic entities; chemical, biological, and physical:

- Chemicals include inorganic substances such as lead, mercury, asbestos, hydrofluoric acid, and chlorine gas, organic compounds such as methyl alcohol, most medications, and poisons from living things.
- Biological toxic entities include those bacteria and viruses that are able to induce disease in living organisms. Biological toxicity can be complicated to measure because the "threshold dose" may be a single organism. Theoretically one virus, bacterium or worm can reproduce to cause a serious infection. However, in a host with an intact immune system the inherent toxicity of the organism is balanced by the host's abilityto fight back; the effective toxicity is then a combination of both parts of the relationship. A similar situation is also present with other types of toxic agents.
- Physically toxic entities include things not usually thought of under the heading of "toxic" by many people: direct blows, concussion, sound and vibration, heat and cold, non-ionizing electromagnetic radiation such as infrared and visible light, and ionizing radiation such as X-rays and alpha, beta, and gamma radiation.
- Toxicity can be measured by the effects on the target (organism, organ, tissue or cell). Because individuals typically have different levels of response to the same dose of a toxin, a population-level measure of toxicity is often used which relates the probability of an outcome for a given individual in a population. One such measure is the LD50. When such data does not exist, estimates are made by comparison to known similar toxic things, or to similar exposures in similar organisms. Then "safety factors" are added to account for uncertainties in data and evaluation processes.

Toxicity studies should be conducted to assess the systemic exposure achieved in animals and its relationship to dose levels and duration of treatment.

### Animal Acclimitization

The laboratory animals that were selected to test the acute toxicity and the diabetes screening were Wistar Albino Rats. They were procured from a seller from Bengaluru city and all the animals are around 3months of age. Their weights ranged from 186gm to 220gm. They were maintained under standard laboratory conditions in their cages and were allowed acclimatize to the conditions. The animals were given the food of a standard pellet feed and fresh water ad libitum. The rats were treated properly and the experiments were progressed as per the guidelines by the Institutional Animal Ethical Committee and the clearance was obtained prior to the experiments. They are maintained in

the plastic cages in the air-conditioned room and the humidity was normally adjusted and the day and light cycles were maintained for every 12hrs.

**Acute Oral Toxicity**

The Extract which was named MESU for Methanol Extract were tested for the acute toxicity screening in the albino rats that are of Wistar strain44. The animals were administered with a standard dose of drug at 2000mg per kg body weight of the rat as per OECD regulations of clause 423. Three rats were selected and the body weights were noted prior to the toxicity study. These were then administered with the extract at the above specified dose. Then the rats were allowed to settle in a separate cage for about 2hrs in observation. The criteria observed were the abnormal behavior of animals like licking the paws and tail, scratching, rubbing and running. The body conditions that were observed for are Tremors, convulsions, abnormal sleep, coma breathing problems and death. The rats were observed for the above conditions for 2 weeks of times and the results were discussed in the following section.Adult Wister rats weighing 160- 200gm were used for the study. The starting dose level of MESU were 2000mg/kg body weight p. o as most of the extracts possess LD50 value more than 200 mg/kg p. o. Dose volume was administered to overnight fasted rats with ad libitum. Food was withheld for further 3- 4 hours after administration of MESU and observed for signs for toxicity.

**Antiobesity Investigation**

The animals that are used to test the antihyperlipidemic activity are the same albino wistar rats. The method used to investigate the antihyperlipidemic activity is the high fat-cholesterol/casein diet induced method75,76. Rats have been acclimatised to normal standard pellet diet during the resting period and the weight gain patterns were noted. HFD that consists of Casein-20%, D, L methionine-0.3%, cornstarch-15%, sucrose-27.7%, cellulose powder-5%, mineral mixture-3.5%, vitamin mixture-1%, corn oil-9.9%, lard oil-17.6% respectively, was administered every day in the morning to the rats except the normal control group animals 77. The grouping of the animals was done as follows.

**Blood Lipid Parameters:**

The rats were anesthetized using ether and after the animals were unconscious, the blood was withdrawn from the retroorbital plexus and the blood was centrifuged at 2500rpm for about 5mins and the serum were separated. The isolated serum samples were analyzed for the lipid parameters like Total cholesterol (TCS), Triglycerides (TGS), Low Density Lipoproteins (LDLS) and Very Low-Density Lipoproteins (VLDLS) and High-Density Lipoproteins (HDLS). The biochemical parameters were analyzed using the commercially available diagnostic kits and as per the standard procedures82.

**Histopathology study**

The pancreas and the adipose tissue from one animal were then dehydrated using a high-grade isopropyl alcohol solution. They are then cleaned with excess amount of xylene and are submerged in the liquid paraffin wax for about 2hrs. This tissue sample was stained with hematoxylin and eosin dye and then subjected for histopathological studies. The tissue blocks were sectioned thinly using a microtome and viewed under a microscope. The pictures were taken using camera attachment in the microscope.

**Enzyme Analysis**

After the duration of 28 days and taking the glucose readings of the rats, they were anesthetized using ether and carefully dissected to obtain the pancreas. The pancreas was isolated from the rats and from all the animals in the group, pancreas of 5 rats was ground to mash in a blender and the tissue homogenate was mixed with 1.5ml of 4.5pH TrisHCl Buffer and allowed to react for 15mins. This was then centrifuged at 400rpm in a centrifuge for about 5mins. The serum that is separated from the blood was collected and stored for the biochemical estimation of the enzymes like Super Oxide Dismuatse (SODS), Catalase (CTS), Lipid peroxidase (LPS), Glutathione Reductase (GRS) and Glutathione Peroxidases (GPXS). The estimations were done as per the standard procedures following in the reference through the commercially available kits in the market83,84.

**Statistical analysis**

The evaluation and comparison of the data was done using the ANOVA with the values in the format of Means and their standard errors for the rats in each group. The unpaired student T-test was applied for comparison between the groups (all groups with normal, all groups with DM induced group). The values that are achieved were considered as significant if the P value is less than 0.01.

**Table 1: Qualitative phytochemical screening of Methanol extract of Stachytarphetaurticifolia.**

| Sl. | Chemical constituents | TEST | RESULTS | Inference |
|---|---|---|---|---|
| **1** | Alkaloids | Mayer's test | - | Absence of Alkaloids |
| | | Dragendorff's test | + | Presence of alkaloids |

| | | | | |
|---|---|---|---|---|
| **2** | Carbohydrates | Barfoed's test | + | Carbohydrates is present |
| **3** | Flavanoids | Alkaline reagent test | + | Flavanoids is present |
| | | Zinc hydrochloride | - | Flavanoids is absent |
| **4** | Anthraquinon glycosides | Test for hydroxyanthraqunion | - | Anthraquninon glycosides is absent |
| **5** | Cardiac glycosides | Baljet's test | - | Absence of cardiac glycosides |
| **6** | Tannins | Ferric chloride test | + | Condansed tannins is present |
| **7** | Gelatin test | Test for catechin | - | Absence of tannins |
| **8** | Steroids & triterpenoids | Salkowski test | + | Presence of steroids & triterpenoids |
| | | Sulfur powder test | + | |

**Table 2: Acute Toxicity test studies of MESU.**

| S. No | Groups | Dose/kg (body weight) p.o | Weight of Rats | | Signs of Toxicity | Onset of Toxicity | Duration of Study |
|---|---|---|---|---|---|---|---|
| | | | Before Test | After Test | | | |
| 1. | MESU | 2000 mg | 160 | 159 | No signs of Toxicity | Nil | 14 days |
| 2. | MESU | 2000 mg | 180 | 181 | No signs of Toxicity | Nil | 14 days |
| 3. | MESU | 2000 mg | 200 | 198 | No signs of Toxicity | Nil | 14 days |
| 4. | MESU | 2000 mg | 170 | 172 | No signs of Toxicity | Nil | 14 days |
| 5. | MESU | 2000 mg | 162 | 162 | No signs of Toxicity | Nil | 14 days |
| 6. | MESU | 2000 mg | 165 | 163 | No signs of Toxicity | Nil | 14 days |

**Table 3: Anti-Obesity effect of MESU on the changes in the body weight of the rats.**

| Groups | Weight on specific day | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 3 | Day 6 | Day 9 | Day 12 | Day 15 | Day 18 | Day 21 |
| Normal Control | 189.85±4.48 | 193.92±0.95 | 194.88±0.46 | 197.98±0.72 | 196.75±0.19 | 198.92±0.17 | 198.47± 0.48 | 196.98± 0.53 |
| High-Fat Diet | 192.98±2.12 | 203.75±1.01 | 210.92±0.84 | 210.92±0.85 | 264.92±1.01 | 284.98±0.22 | 304.78± 0.75 | 312.95± 0.87 |
| Orlistat | 191.88±1.96 | 196.32±0.79 | 196.75±0.75 | 196.75±0.75 | 192.42±0.43 a | 195.78±0.09 a | 190.05± 0.50a | 189.88± 0.71a |
| MESU-250 | 190.85±3.45 | 195.92±0.59 | 197.21±0.58 | 197.21±0.58 | 204.12±0.65 a | 201.38±0.22 a | 207.34± 0.46a | 210.12± 0.41a |
| MESU-500 | 191.78±2.01 | 193.45±0.61 | 194.60±0.60 | 194.60±0.60 | 193.75±0.08 a | 195.02±0.09 a | 192.42± 0.36a | 190.65± 0.52a |

**Table 4: Anti-Obesity effect of MESU and MESU on the lipid profile of the rats.**

| Group treatment | TC's (mg/dL) | TG's (mg/dL) | LDL (mg/dL) | VLDL (mg/dL) | HDL (mg/dL) | Lee index | BMI |
|---|---|---|---|---|---|---|---|
| Normal saline | 74.98±0.95 | 87.92±0.63 | 33.98±0.14 | 22.45±0.12 | 31.60±0.36 | 41.12±0.95 | 0.9987±0.046 |
| High Fat Diet | 214.58±1.26 | 137.89±0.39 | 148.98±0.60 | 29.88±0.11 | 21.85±0.22 | 48.45±1.14 | 1.6183±0.074 |
| Orlistat | 76.92±0.38a | 90.74±0.45a | 47.98±0.56a | 23.08±0.24a | 28.98±0.23a | 38.98±0.60a | 0.8852±0.027a |
| MESU-250 | 135.78±0.82ab | 106.12±0.73a | 97.98±0.72ab | 29.15±0.26b | 26.45±0.14ab | 42.72±0.99 | 1.1002±0.050 |
| MESU-500 | 104.92±0.80ab | 125.02±0.97 | 79.85±0.31ab | 25.74±0.14 a | 29.12±0.14 a | 40.28±0.71a | 0.9475±0.033a |

**Table 5: Anti-obesity effect of MESU and MESU on the Antioxidant Enzyme levels.**

| Group treatment | Superoxide Dismutase Units/mg | Catalase Units/mg | Lipid Peroxidation Nmol/mg | Glutathione Reductase Units/mg | Glutathione Peroxidase Units/mg |
|---|---|---|---|---|---|
| Normal saline | 5.3125±0.067 | 24.185±0.122 | 2.6987±0.070 | 31.572±0.356 | 26.228±0.251 |
| induced group | 14.508±0.148 | 17.692±0.101 | 7.2789±0.096 | 8.072±0.227 | 15.975±0.405 |
| Orlistat | 6.6989±0.148a | 21.056±0.101a | 4.3827±0.065a b | 19.654±0.134a b | 21.975±0.295b |
| MESU-250 | 7.7225±0.258 a | 19.498±0.137b | 5.6842±0.109 b | 23.825±0.338 a b | 22.592±0.360b |

| | | | | | |
|---|---|---|---|---|---|
| MESU-500 | 6.8123±0.076 a | 21.498±0.098 a | 3.1124±0.176 a | 29.352±0.098 a | 25.794±0.350 a |

**RESULTS AND DISCUUSION**

**Preliminary phytochemical screening of extracts**

Qualitative phytochemical screening was carried out using several tests and results revealed that Methanol extract of *Stachytarphetaurticifolia.*contains phenols, flavonoids and absence of alkaloids, carbohydrates and steroids & triterpenoids.

**ACUTE TOXICITY STUDIES**

The acute toxicity study was performed using the highest standard dose. There was no change in weight and animal behavior.

Eyes, skin and tails are normal and no abnormal signs were noted. Animal remained healthy and active till the 14th day. There was no mortality and morbidity in the rats with the treatment with extracts. As it was found safe at 2000mg/kg of the extract, the effective dose ED50 was established as 250mg/kg and the higher dose 500mg/kg was also tested for anti-diabetic activity and anti-obesity activity.

The body weight of the rats before and after administration were noted that there is no changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also no sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also was not seen. In further study there was no toxicity/ death were observed at these levels.

**ANTI-OBESITY ACTIVITY**

The changes in the body weight also was significant in all the groups when compared to the obesity induced group. With the high fat diet in the batch-II, the weight gain was very significant and the normal group of rats. The weight gain in normal control of rats was not significant and the animals gained just 5gm of weight which was normal in the rats with no physical exercise. This was compared significant compared to the negative control group of rats which gained almost 50% more weight due to the high fat cholesterol diet induced method. The weight gain was significant in the group of animals that received the methanol extract at a dose of 500mg/kg body weight. The weight gain was not prevented by the standard drug which concentrates the lowering of the lipids in the body by just lowering the blood lipid levels but not cause any changes in the body weight.The BMI of the normal groups measured in the normal range with a minor change that is due to lack of physical exercise and the groups that is fed with high fat diet have gained a lot of BMI which confirms that the rats became obese with the HFD. The standard drug Orlistat was able to lower the BMI value as it lowered the body weight gain. The extracts showed a similar effect as the weight gain was prevented by the methanol extract at a higher dose of 500mg/kg then the water extract at a same dose and the extracts showed a dose dependent raise in the activity.

This study evaluates the impact of MESU at different doses (250 mg/kg and 500 mg/kg) on lipid profile and obesity markers, comparing it to a high-fat diet (HFD) group, an Orlistat-treated group, and a normal saline control group. The parameters assessed include total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL), Lee index, and body mass index (BMI). These markers provide insight into the effectiveness of MESU in managing diet-induced obesity and dyslipidemia. Lipid abnormalities are a hallmark of obesity and metabolic syndrome, often characterized by elevated total cholesterol, LDL, VLDL, and triglycerides, alongside reduced HDL levels. The normal saline group serves as the baseline, reflecting healthy lipid levels and normal metabolic function. The HFD group, on the other hand, shows a dramatic increase in TC (214.58 mg/dL), LDL (148.98 mg/dL), TG (137.89 mg/dL), and VLDL (29.88 mg/dL), alongside a significant decrease in HDL (21.85 mg/dL). These changes indicate dyslipidemia and a high risk of cardiovascular disease, confirming that a high-fat diet induces significant metabolic disturbances. Treatment with Orlistat, a well-known lipase inhibitor, effectively lowered TC, LDL, and TG levels, while improving HDL levels.

The Orlistat group exhibited a significant reduction in TC (76.92 mg/dL), LDL (47.98 mg/dL), and TG (90.74 mg/dL), with an increase in HDL to 28.98 mg/dL. These findings align with Orlistat's established mechanism of action, where it blocks fat absorption in the intestine, leading to improved lipid metabolism and weight regulation. Among the MESU-treated groups, both MESU-250 and MESU-500 demonstrated lipid-lowering effects, though in a dose-dependent manner. MESU-250 resulted in a moderate reduction in TC (135.78 mg/dL) and LDL (97.98 mg/dL), with slight improvements in TG (106.12 mg/dL) and HDL (26.45 mg/dL). While these results indicate some protective effects, the higher dose (MESU-500) exhibited stronger lipid-lowering effects, with TC reduced to 104.92 mg/dL, LDL to 79.85 mg/dL, and TG to 125.02 mg/dL. Notably, HDL levels in the MESU-500 group (29.12 mg/dL) were significantly higher than in the MESU-250 group, suggesting an improved atherogenic index and cardiovascular protection. These findings imply that MESU may modulate lipid metabolism, possibly by enhancing lipid clearance pathways, reducing lipid absorption, or promoting the breakdown of stored fats.The Lee index and BMI are widely used markers for assessing obesity and metabolic status. The HFD group showed a significant increase in BMI (1.6183) and Lee index (48.45),

confirming severe obesity induced by excessive fat intake. The accumulation of body fat and increased BMI in this group correlate with the observed lipid abnormalities, further reinforcing the detrimental effects of a high-fat diet on metabolic health. In contrast, Orlistat-treated rats exhibited a significant reduction in BMI (0.8852) and Lee index (38.98), supporting its anti-obesity effects. By blocking fat absorption, Orlistat prevents excessive fat accumulation and promotes weight stabilization, making it a standard reference treatment for comparison.The MESU-treated groups also showed dose-dependent weight regulation effects. MESU-250 resulted in a BMI of 1.1002 and a Lee index of 42.72, indicating moderate protection against weight gain. However, MESU-500 provided stronger anti-obesity benefits, with a BMI of 0.9475 and a Lee index of 40.28, suggesting improved weight regulation and metabolic balance. These results suggest that MESU, particularly at a higher dose, may exert anti-obesity effects, possibly through mechanisms such as fat oxidation, improved lipid metabolism, and enhanced energy expenditure.

The mechanism of the weight gain and the causation of the Hyperlipidemia due to the high fat diet induced with cholesterol was clearly understood earlier. The feeding of cholesterol to the rats elevated the serum levels of unhealthy fats and lipids and tissue cholesterol causing the metabolic disorders in the rats. The saturated fats along with the cholesterol were significantly induced for the elevated levels. This elevation of the saturated fats concentration in the blood was facilitated by the high fat diet85. They cause some heart problems like atherosclerosis and the congestive heart failure in the population of the world86. The elevation of the LDL and VLDL means the elevation in the saturated fats. This was the main reason for most of the heart attacks of the people87. The high-density lipids were lowered due to the inability to process the lipids and failure in the successful conversion of the LDL to HDL which are considered safer fats. This leads to the increase in the total cholesterol, lipids and decrease in the HDL. The histopathological study of the tissue slides were shown in the figure 7.2 with marked regeneration of the adipose tissue is seen in the extract treated groups with MESU-500mg/kg and the total degeneration of adipose tissue and the growth of the lipid globule in to a rapid size was evident in the slide b which is the HFD induced hyperlipidemia group.The present study examines the effects of MESU (250 mg/kg and 500 mg/kg) on antioxidant enzyme activity and lipid peroxidation levels in comparison to a normal saline group, an induced oxidative stress group, and an Orlistat-treated group. The parameters evaluated include superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (LPO), glutathione reductase (GR), and glutathione peroxidase (GPx). These biomarkers provide insights into oxidative stress levels and the ability of MESU to enhance cellular antioxidant defenses.Oxidative stress occurs when reactive oxygen species (ROS) production exceeds the body's natural antioxidant defense mechanisms, leading to cellular damage, inflammation, and metabolic dysfunction. SOD, catalase, and glutathione-related enzymes play a crucial role in neutralizing ROS, protecting cells from oxidative damage. Lipid peroxidation, on the other hand, serves as an indicator of oxidative damage to cellular membranes, with higher levels correlating to greater oxidative stress.The oxidative stress-induced group exhibited significant alterations in antioxidant enzyme levels, with a marked increase in lipid peroxidation (7.2789 nmol/mg), indicating severe oxidative damage. SOD levels (14.508 U/mg) were highly elevated, suggesting a compensatory response to excessive superoxide radicals. However, catalase activity was significantly reduced (17.692 U/mg), implying an impaired ability to decompose hydrogen peroxide into water and oxygen. The glutathione system was also severely affected, with glutathione reductase (8.072 U/mg) and glutathione peroxidase (15.975 U/mg) showing drastic reductions, indicating depleted antioxidant reserves and compromised detoxification mechanisms. These findings confirm that oxidative stress leads to severe antioxidant system dysfunction, increasing cellular vulnerability to damage.The Orlistat-treated group showed partial restoration of antioxidant defenses, with a decrease in lipid peroxidation levels (4.3827 nmol/mg) and improved SOD (6.6989 U/mg) and catalase (21.056 U/mg) activity. Glutathione-related enzyme levels also showed improvements, with glutathione reductase increasing to 19.654 U/mg and glutathione peroxidase rising to 21.975 U/mg. These findings indicate that Orlistat may possess mild antioxidant properties, possibly by reducing lipid absorption and limiting ROS production from excessive fat metabolism.Both MESU-250 and MESU-500 demonstrated significant improvements in antioxidant enzyme levels and reductions in oxidative stress markers, with MESU-500 showing superior effects. MESU-250 showed a moderate antioxidant response, with SOD (7.7225 U/mg) and catalase (19.498 U/mg) activity improving compared to the induced group, though still lower than Orlistat. Lipid peroxidation levels remained relatively high at 5.6842 nmol/mg, suggesting partial protection against oxidative stress. However, glutathione reductase (23.825 U/mg) and glutathione peroxidase (22.592 U/mg) were significantly restored, indicating an improvement in glutathione metabolism and cellular detoxification capacity.MESU-500 provided stronger antioxidant effects, with SOD (6.8123 U/mg) and catalase (21.498 U/mg) approaching normal saline levels. Lipid peroxidation levels were significantly reduced (3.1124 nmol/mg), demonstrating MESU-500's superior ability to prevent oxidative damage. The glutathione system was also highly restored, with glutathione reductase (29.352 U/mg) and glutathione peroxidase (25.794 U/mg) showing the highest values across all treated groups, suggesting that MESU-500 effectively enhances glutathione recycling and detoxification mechanisms..

## DISCUSSION

Obesity has become a global health concern with its associated complications such as cardiovascular diseases, diabetes, and metabolic disorders. Natural products have gained significant attention in recent years for their potential anti-obesity effects. In this study, we investigated the anti-obesity potential of the Methanol Extract of Stachytarphetaurticifolia (MESU) using in vivo models. The objective was to explore the possible mechanisms underlying their therapeutic actions.Before delving into the potential mechanisms of action, it is essential to establish the safety profile of the extracts. Acute toxicity studies were performed using the highest standard dose (2000 mg/kg body weight) of MESU. No signs of toxicity were observed in the animals throughout the 14-day study period. These findings indicate that the extracts have a favorable safety profile, allowing for further investigation of their anti-obesity effects.The study evaluated the effects of MESU on body weight, lipid profile, and antioxidant enzyme levels in high-fat diet-induced obese rats. The results demonstrated significant changes in body weight and lipid profile parameters compared to the induced group, highlighting the potential anti-obesity effects of both extracts. The induced group exhibited a progressive increase in body weight, while treatment with MESU showed a notable reduction in weight gain. This finding suggests that both extracts possess anti-obesity properties by inhibiting weight gain in the high-fat diet-induced obese rats.The dysregulation of lipid metabolism is a key contributor to obesity. MESU treatment significantly improved the lipid profile by reducing total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) levels, while increasing high-density lipoprotein (HDL) levels. These alterations indicate a potential regulatory effect of the extracts on lipid metabolism, thereby preventing lipid accumulation and reducing the risk of obesity-related complications. Obesity is associated with oxidative stress, which contributes to the development of metabolic disorders. MESU treatment restored the antioxidant enzyme levels, including superoxide dismutase (SOD), catalase, lipid peroxidation, glutathione reductase, and glutathione peroxidase, towards normal levels. These findings suggest that the extracts possess antioxidative properties, potentially mitigating oxidative stress associated with obesity.The observed anti-obesity effects of MESU can be attributed to their active constituents and their interactions with various metabolic pathways. Several potential mechanisms of action can be proposed based on existing scientific literature: The extracts may modulate lipid metabolism by inhibiting lipogenesis and promoting lipolysis. This could be mediated through the regulation of key enzymes involved in lipid synthesis, such as fatty acid synthase (FAS) and hormone-sensitive lipase (HSL).MESU may exhibit appetite-suppressing effects, leading to reduced food intake. This could be achieved by modulating neuropeptides involved in appetite regulation, such as leptin, ghrelin, and neuropeptide Y (NPY). The extracts may inhibit the differentiation of preadipocytes into mature adipocytes, thereby reducing adipogenesis and preventing adipocyte hypertrophy. This could be mediated through the modulation of peroxisome proliferator-activated receptor-gamma (PPAR-γ) and CCAAT/enhancer-binding protein alpha (C/EBP-α) signaling pathways. MESU may stimulate thermogenesis and increase energy expenditure, leading to a negative energy balance. This could involve the activation of brown adipose tissue (BAT) and the upregulation of uncoupling protein 1 (UCP1).

## CONCLUSION

In conclusion, the findings of this study shed light on the remarkable anti-obesity potential of the Methanol Extract of Stachytarphetaurticifolia (MESU). Through meticulous experimentation and rigorous analysis, we have unraveled the therapeutic properties of these extracts in combating obesity. Our acute toxicity studies provided reassuring evidence of the safety of MESU and MESU, as no signs of toxicity were observed even at the highest standard dose. This crucial aspect sets a solid foundation for further exploration of their therapeutic benefits. The results of our comprehensive evaluation unveiled the profound impact of MESU on various parameters related to obesity. Notably, both extracts demonstrated a significant reduction in weight gain, highlighting their potential as anti-obesity agents. By effectively modulating lipid metabolism, these extracts exhibited a remarkable ability to lower total cholesterol, triglycerides, and LDL levels while increasing HDL levels, thus mitigating the risk of obesity-related complications. Furthermore, the extracts exhibited a remarkable ability to restore antioxidant enzyme levels, effectively combating the oxidative stress associated with obesity. This antioxidative property holds immense promise in preventing the development of metabolic disorders and improving overall health outcomes. While our study provides compelling evidence of the anti-obesity effects of MESU, it is important to acknowledge that the underlying mechanisms of action are complex and multifaceted. Further investigations are warranted to elucidate the active constituents responsible for these effects and to unravel the precise molecular pathways involved. The present study serves as a stepping stone toward harnessing the therapeutic potential of Stachytarphetaurticifolia extracts for the management of obesity. It highlights the importance of exploring natural products as potential alternatives or adjuncts to conventional anti-obesity treatments. In conclusion, the remarkable anti-obesity effects exhibited by MESU underscore the significance of Stachytarphetaurticifolia as a potential source of novel anti-obesity therapeutics. By offering unique mechanisms of action and an excellent safety profile, these extracts hold promise for future drug development efforts. Our research contributes to the growing body of knowledge on natural

products and paves the way for further investigations and potential clinical applications in the battle against obesity.

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