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Research Article

Pharmacological Evaluation of Anti-Obesity Activity with Etanolic Extract of *Hygrophila auriculata* in Rats

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ABSTRACT

Obesity is a chronic metabolic disorder characterized by excessive accumulation of body fat and is associated with an increased risk of cardiovascular diseases, type 2 diabetes mellitus, dyslipidemia, hypertension, and other metabolic complications. Despite the availability of pharmacological interventions, the long-term use of anti-obesity drugs is often limited by adverse effects, necessitating the search for safer and more effective natural alternatives. The present study was undertaken to evaluate the anti-obesity activity of the ethanolic extract of *Hygrophila auriculata* in high-fat diet (HFD)-induced obese Wistar albino rats.Preliminary phytochemical screening of the ethanolic extract revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, glycosides, proteins, carbohydrates, amino acids, and fixed oils. Obesity was induced by feeding rats a high-fat diet for 6–8 weeks. The animals were divided into six groups: normal control, HFD control, standard (Orlistat, 10 mg/kg), and three treatment groups receiving the ethanolic extract of *Hygrophila auriculata* at doses of 100, 200, and 400 mg/kg orally for 28 consecutive days. At the end of the treatment period, body weight, body mass index (BMI), Lee index, serum lipid profile [total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL)], and oxidative stress markers [malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH)] were evaluated. Administration of the high-fat diet produced a significant increase in body weight, BMI, Lee index, TC, TG, LDL, VLDL, and MDA levels, along with a significant reduction in HDL, SOD, CAT, and GSH levels compared with the normal control group. Treatment with the ethanolic

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extract significantly and dose-dependently reduced body weight gain, BMI, Lee index, TC, TG, LDL, VLDL, and MDA levels while significantly increasing HDL, SOD, CAT, and GSH levels. Among the tested doses, the 400 mg/kg extract produced the most pronounced effects and showed efficacy comparable to the standard drug, Orlistat.the present study demonstrate that the ethanolic extract of *Hygrophila auriculata* possesses significant anti-obesity, antihyperlipidemic, and antioxidant activities in high-fat diet-induced obese rats. These beneficial effects are likely attributable to the synergistic action of its phytoconstituents, particularly flavonoids, phenolic compounds, saponins, terpenoids, and phytosterols, which regulate lipid metabolism and protect against oxidative stress. The study provides scientific evidence supporting the traditional use of *Hygrophila auriculata* in the management of obesity and suggests that it may serve as a promising natural therapeutic agent for the prevention and treatment of obesity and its associated metabolic disorders. However, further studies are required to isolate the active constituents, elucidate the underlying molecular mechanisms, and establish its long-term safety and clinical efficacy.

INTRODUCTION

OBESITY

Obesity is a chronic, multifactorial disease characterized by excessive accumulation of body fat that negatively affects health and increases the risk of numerous metabolic and cardiovascular complications. It is generally defined using the Body Mass Index (BMI), where a BMI of 30 kg/m² or higher is classified as obesity. However, obesity is now recognized not just as a lifestyle condition but as a complex disease involving neuroendocrine, metabolic, genetic, and environmental factors.The development of obesity results from a long-term imbalance between energy intake and energy expenditure, leading to increased storage of excess calories in adipose tissue. This process is regulated by the central nervous system, particularly the hypothalamus, which controls appetite and satiety through hormonal signals such as leptin, insulin, and ghrelin. In obesity, these regulatory mechanisms become disrupted, leading to increased hunger, reduced satiety, and continuous fat accumulation.

Obesity is strongly associated with chronic low-grade inflammation and oxidative stress, as adipose tissue acts as an active endocrine organ. It secretes various pro-inflammatory cytokines and adipokines that contribute to metabolic dysfunction and insulin resistance. As a result, obesity is considered a key underlying factor in many non-communicable diseases.The disease is closely linked with serious health conditions, including Type 2 Diabetes, Hypertension, cardiovascular diseases, dyslipidaemia, stroke, non-alcoholic fatty liver disease, osteoarthritis, and certain cancers. It also has psychological impacts, including depression, anxiety, and reduced quality of life.Due to its rapidly increasing global prevalence, obesity has become a major public health challenge. Its management requires a multidisciplinary approach involving lifestyle modification, pharmacological interventions, and in some cases, surgical procedures. Understanding obesity as a disease is essential for developing effective prevention and treatment strategies [1-3].

HISTORY OF OBESITY

Obesity is a condition that has been recognized since ancient times, but its understanding has evolved significantly over history. In early civilizations such as Egypt, Greece, and Rome, obesity was often associated with wealth, prosperity, and social status because food scarcity was common, and excess body weight indicated abundance. However, early physicians like Hippocrates were among the first to observe that excessive body fat was linked to poor health and reduced life expectancy, emphasizing the importance of diet and physical activity. During the medieval period, obesity continued to be viewed both as a symbol of affluence and as a medical imbalance in traditional systems of medicine such as Ayurveda and Unani, where it was associated with improper digestion and lifestyle habits. By the 19th century, obesity began to be more systematically studied as a medical condition rather than a social trait, with increasing recognition of its relationship to metabolism and lifestyle. The 20th century marked a major advancement with the introduction of the Body Mass Index (BMI) by Adolphe Quetelet, which provided a standardized method for classifying body weight. During this period, obesity was also identified as a major risk factor for diseases such as Type 2 Diabetes and Hypertension, leading to extensive research into its hormonal, genetic, and metabolic causes. In the modern

era, obesity is now recognized as a chronic, multifactorial disease and a global public health challenge, driven by complex interactions between genetics, environment, lifestyle, and metabolic regulation [4].

Obesity in Medicinal Plants

Medicinal plants have been extensively used in traditional medicine systems for the management of obesity and related metabolic disorders. Obesity is a complex disease involving disturbances in lipid metabolism, energy balance, hormonal regulation, and chronic inflammation. Due to the limitations and side effects associated with synthetic anti-obesity drugs, there has been increasing interest in plant-based therapies as safer and more effective alternatives for long-term management.Herbal medicines contain a wide range of bioactive phytoconstituents such as flavonoids, alkaloids, saponins, tannins, glycosides, terpenoids, and phenolic compounds. These compounds act through multiple mechanisms to combat obesity.

They may reduce appetite, inhibit fat absorption, enhance lipid metabolism, stimulate energy expenditure, and improve glucose utilization. Some plant constituents also modulate key enzymes such as pancreatic lipase, which is responsible for dietary fat digestion, thereby reducing fat accumulation in the body.Another important mechanism of medicinal plants in obesity management is the regulation of adipogenesis (formation of fat cells). Certain phytochemicals inhibit the differentiation of pre-adipocytes into mature adipocytes, thereby reducing fat storage. In addition, many plant extracts exhibit antioxidant and anti-inflammatory properties, which help in reducing oxidative stress and chronic inflammation associated with obesity.Medicinal plants also play a role in improving lipid profiles by lowering total cholesterol, triglycerides, and low-density lipoprotein (LDL) levels while increasing high-density lipoprotein (HDL) levels. This lipid-lowering effect is crucial in preventing obesity-related complications such as Type 2 Diabetes and Hypertension.

Traditional systems of medicine, including Ayurveda, Siddha, and Unani, have long utilized herbal formulations for weight management. Plants such as *Camellia sinensis* (green tea), *Garcinia cambogia*, *Curcuma longa* (turmeric), *Trigonella foenum-graecum* (fenugreek), *Nigella sativa*, and *Hygrophila auriculata* are widely studied for their anti-obesity potential.In modern pharmacological research, these plants are evaluated using in vitro assays, in vivo animal models, and biochemical parameters to validate their efficacy. Experimental studies in obese rat models are particularly important for assessing body weight reduction, lipid profile improvement, and histopathological changes in adipose and liver tissues.medicinal plants offer a promising multi-targeted approach for obesity management by acting on various metabolic pathways simultaneously. Their natural origin, lower side-effect profile, and therapeutic potential make them valuable candidates for developing novel anti-obesity drugs [5-7].

Types of Obesity

Obesity can be classified in different ways based on fat distribution, cause, and severity, which helps in understanding its clinical significance and associated risks. Based on fat distribution, obesity is mainly divided into central (abdominal or visceral) obesity and peripheral (gynoid) obesity. Central obesity is characterized by excess fat accumulation around the abdomen and internal organs and is strongly associated with metabolic complications such as Type 2 Diabetes and Hypertension, along with cardiovascular diseases, whereas peripheral obesity involves fat deposition mainly in the hips and thighs and is comparatively less metabolically harmful. Based on etiology, obesity is classified as primary obesity, which is mainly due to lifestyle factors such as unhealthy diet, sedentary behavior, and environmental influences, and secondary obesity, which results from underlying medical conditions or long-term use of certain medications like corticosteroids and antidepressants. Additionally, based on severity, obesity is categorized using Body Mass Index (BMI) into overweight (25–29.9 kg/m²), Class I obesity (30–34.9 kg/m²), Class II obesity (35–39.9 kg/m²), and Class III or morbid obesity (≥40 kg/m²). Overall, these classifications help in assessing disease risk and guiding appropriate management strategies [8].

Central (Abdominal/Visceral) Obesity

Central or abdominal (visceral) obesity is a type of obesity characterized by excessive accumulation of fat around the abdominal region and internal organs such as the liver, pancreas, and intestines. It is commonly referred to as "apple-shaped" obesity due to the concentration of fat in the upper body. This type of fat deposition is metabolically active and is strongly associated with insulin resistance, chronic inflammation, and hormonal imbalance. Central obesity is considered more dangerous than peripheral obesity because visceral fat releases pro-inflammatory cytokines and free fatty acids that disrupt normal metabolic processes. It is a major risk factor for the development of serious conditions such as Type 2 Diabetes, Hypertension, cardiovascular diseases, and non-alcoholic fatty liver disease. It is often linked to sedentary lifestyle, unhealthy dietary habits, stress, and genetic predisposition. Measurement of waist circumference is commonly used to assess central obesity and evaluate associated health risks [9].

Peripheral Obesity

Peripheral obesity is a type of obesity in which excess fat is mainly distributed in the peripheral parts of the body, such as the hips, thighs, and buttocks. It is often described as a "pear-shaped" body pattern. Unlike central obesity, fat accumulation in peripheral obesity is predominantly subcutaneous rather than visceral, meaning it is stored beneath the skin rather than around internal organs. This type of fat distribution is generally considered less metabolically harmful compared to abdominal obesity, as it has a weaker association with metabolic complications. However, it can still lead to physical discomfort, reduced mobility, and joint-related problems due to increased body weight. Peripheral obesity is influenced by genetic factors, hormonal balance—particularly estrogen in females—and lifestyle factors such as physical inactivity and excessive calorie intake. Although it carries a lower risk for conditions like Type 2 Diabetes and Hypertension compared to central obesity, it still requires proper dietary control and physical activity for management and prevention of further health complications.

Primary Obesity

Primary obesity is the most common type of obesity, which develops mainly due to an imbalance between energy intake and energy expenditure without any underlying medical or pathological cause. It is primarily associated with lifestyle factors such as excessive consumption of high-calorie foods, increased intake of fats and sugars, irregular eating habits, and a sedentary lifestyle with reduced physical activity. Genetic predisposition may also contribute to primary obesity by influencing appetite regulation, metabolism, and fat storage patterns. In this condition, excess calories are stored as fat in adipose tissues over a prolonged period, leading to gradual weight gain. Primary obesity is strongly influenced by environmental and behavioral factors such as urbanization, stress, and lack of awareness about healthy dietary practices. Although it is not caused by a specific disease, primary obesity significantly increases the risk of metabolic disorders like Type 2 Diabetes and Hypertension, along with cardiovascular complications and other chronic health conditions[10-11].

Secondary Obesity

Secondary obesity is a type of obesity that develops as a consequence of underlying medical conditions, hormonal disorders, or prolonged use of certain medications, rather than being caused primarily by lifestyle factors alone. It occurs when normal metabolic or endocrine functions are disrupted, leading to abnormal fat accumulation in the body. Common medical causes include endocrine disorders such as hypothyroidism, Cushing's syndrome, and polycystic ovary syndrome (PCOS), which alter hormonal balance, reduce metabolic rate, and promote fat storage. In addition, neurological conditions and genetic syndromes may also contribute to secondary obesity in some cases.

Certain medications can also induce weight gain and lead to secondary obesity. Drugs such as corticosteroids, antidepressants, antipsychotics, and some antiepileptic agents may increase appetite, alter metabolism, or promote fluid and fat retention, resulting in gradual weight gain over time. Unlike primary obesity, secondary obesity has a clear identifiable cause,

and management often requires treating the underlying disorder along with lifestyle modification.

Secondary obesity is associated with an increased risk of metabolic complications, including Type 2 Diabetes and Hypertension, as well as cardiovascular diseases and other long-term health problems. Early diagnosis and appropriate medical intervention are essential for effective management and prevention of complications [12].

Complications of Obesity

Obesity is associated with a wide range of serious health complications affecting multiple organ systems, making it a major risk factor for both morbidity and mortality. One of the most important metabolic complications is insulin resistance, which can progress to Type 2 Diabetes due to impaired glucose utilization and pancreatic β-cell dysfunction. Cardiovascular complications are also highly prevalent, with obesity significantly increasing the risk of Hypertension, atherosclerosis, coronary artery disease, stroke, and heart failure.

Obesity is also strongly linked to dyslipidemia, characterized by elevated triglycerides, increased low-density lipoprotein (LDL), and reduced high-density lipoprotein (HDL), which further contributes to cardiovascular risk. In the liver, excess fat accumulation can lead to non-alcoholic fatty liver disease (NAFLD), which may progress to steatohepatitis and liver dysfunction. Respiratory complications such as obstructive sleep apnea and reduced lung capacity are also common due to fat deposition around the airway and chest wall.

In the musculoskeletal system, increased body weight places excessive stress on joints, leading to osteoarthritis, particularly in weight-bearing joints such as the knees and hips. Obesity is also associated with hormonal imbalances, reproductive dysfunction, and an increased risk of certain cancers, including breast, colon, and endometrial cancers. In addition to physical health effects, obesity has psychological consequences such as depression, anxiety, low self-esteem, and social stigma, which further reduce quality of life. Overall, obesity leads to multisystem complications that significantly impact health and longevity [13-14].

Public Health Importance of Obesity

Obesity is a major public health concern worldwide due to its rapidly increasing prevalence and its strong association with numerous chronic diseases. It is considered a key risk factor for non-communicable diseases such as Type 2 Diabetes, Hypertension, cardiovascular diseases, stroke, and certain types of cancer. The rising burden of obesity significantly increases healthcare costs, as long-term management of its complications requires continuous medical care, medications, and hospital services.

From a public health perspective, obesity affects individuals across all age groups, including children, adolescents, and adults, making it a life-course health issue. The condition is closely linked to modern lifestyle changes such as sedentary behavior, unhealthy dietary patterns, urbanization, and increased consumption of processed foods. This makes it not only a medical problem but also a societal and environmental issue.

Obesity also contributes to reduced productivity, increased absenteeism from work, and decreased quality of life, thereby impacting economic development at both individual and national levels. Furthermore, it places a significant burden on healthcare systems, particularly in developing countries where resources are already limited.Due to these factors, obesity prevention and control have become major priorities in public health policies. Strategies such as promoting healthy diets, encouraging physical activity, improving awareness, and implementing community-based interventions are essential to reduce the growing burden of obesity globally [15-16].

Etiology of Obesity

The etiology of obesity is multifactorial and results from a complex interaction of genetic, environmental, behavioral, hormonal, and metabolic factors that lead to an imbalance between energy intake and energy expenditure. One of the major contributing factors is genetic predisposition, where inherited traits influence appetite regulation, fat storage, and metabolic rate, increasing susceptibility to weight gain. Dietary habits also play a crucial role, as excessive consumption of high-calorie foods rich in fats, sugars, and refined

carbohydrates, along with frequent intake of processed and fast foods, promotes positive energy balance. Physical inactivity further contributes to obesity by reducing energy expenditure, especially in individuals with sedentary lifestyles and minimal physical exercise. Hormonal and metabolic disorders such as hypothyroidism, insulin resistance, and polycystic ovary syndrome (PCOS) can also disrupt normal metabolism and promote fat accumulation. Psychological factors including stress, depression, and emotional eating behavior are important contributors, often associated with increased cortisol levels that favor abdominal fat deposition. Environmental and socioeconomic factors such as urbanization, lack of awareness, and easy availability of energy-dense foods further increase the risk of obesity. In addition, certain medications like corticosteroids and antidepressants may lead to weight gain as a side effect. Overall, obesity arises from multiple interacting factors that disrupt normal energy balance and lead to excessive fat accumulation in the body [17].

Dietary Habits

Dietary habits play a crucial role in the development and progression of obesity. Obesity commonly arises when there is a sustained intake of high-calorie foods that exceeds the body's energy requirements. Modern dietary patterns, characterized by increased consumption of fast foods, processed snacks, sugary beverages, and foods rich in saturated fats and refined carbohydrates, significantly contribute to positive energy balance and fat accumulation. Frequent snacking, irregular meal patterns, and oversized portions further worsen calorie overload. Low intake of dietary fiber, fruits, and vegetables reduces satiety and promotes overeating. Additionally, excessive sugar consumption can lead to rapid spikes in blood glucose and insulin levels, encouraging fat storage over time. Poor dietary choices, combined with sedentary lifestyle habits, are one of the strongest modifiable risk factors for obesity and related metabolic disorders.

Physical Inactivity

Physical inactivity is one of the major contributing factors in the development of obesity. It refers to a sedentary lifestyle with insufficient levels of physical movement or exercise to maintain energy balance. When physical activity is low, the body expends fewer calories, and the excess energy obtained from food is stored as fat, leading to gradual weight gain. Modern lifestyle changes such as prolonged sitting, increased screen time, desk-based work, and reduced outdoor activities have significantly increased sedentary behavior. Lack of regular exercise also slows down metabolic rate, reduces muscle mass, and decreases overall energy expenditure, further promoting fat accumulation. In addition, physical inactivity is associated with poor lipid metabolism and reduced insulin sensitivity, which can worsen obesity-related metabolic complications. Therefore, regular physical activity is essential for maintaining healthy body weight and preventing obesity.

Hormonal and Metabolic Disturbances

Hormonal and metabolic disturbances play a significant role in the development and progression of obesity by disrupting normal regulation of appetite, energy balance, and fat metabolism. The hypothalamus controls hunger and satiety through hormonal signals such as leptin, insulin, and ghrelin. In obesity, leptin resistance commonly occurs, leading to impaired satiety signaling and increased food intake despite sufficient energy stores. Similarly, insulin resistance reduces glucose utilization and promotes fat deposition in adipose tissue, contributing to weight gain and metabolic dysfunction. Elevated ghrelin levels may further stimulate appetite, increasing caloric intake. Metabolic disorders such as hypothyroidism can also slow basal metabolic rate, reducing energy expenditure and favoring fat accumulation. Additionally, conditions like polycystic ovary syndrome (PCOS) are associated with hormonal imbalance, insulin resistance, and increased risk of obesity. These hormonal and metabolic abnormalities collectively disrupt energy homeostasis and significantly contribute to the pathogenesis of obesity [18].

Psychological Factors

Psychological factors are important contributors to the development and progression of obesity, as they influence eating behavior, lifestyle choices, and hormonal responses. Conditions such as stress, anxiety,

depression, and emotional distress often lead to "emotional eating," where individuals consume high-calorie or comfort foods as a coping mechanism. Chronic stress is associated with increased secretion of cortisol, a hormone that promotes fat accumulation, particularly in the abdominal region. Depression and low mood can reduce motivation for physical activity and encourage sedentary behavior, further contributing to weight gain. Sleep disturbances and poor sleep quality, which are often linked with psychological stress, can also disrupt appetite-regulating hormones such as leptin and ghrelin, leading to increased hunger and overeating. Over time, these psychological and behavioral patterns significantly contribute to positive energy imbalance and the development of obesity.

Environmental and Socioeconomic Factors

Environmental and socioeconomic factors play a significant role in the development of obesity by influencing lifestyle patterns, dietary choices, and physical activity levels. Modern urban environments often promote sedentary behavior due to increased use of transportation, desk-based occupations, and reduced opportunities for physical exercise. Easy availability and aggressive marketing of fast foods, processed snacks, and sugar-rich beverages further encourage unhealthy eating habits. In many communities, lack of safe public spaces for physical activity also discourages regular exercise. Socioeconomic status is another important determinant, as individuals from lower-income groups may have limited access to healthy, nutritious foods and may rely more on inexpensive, calorie-dense diets. Additionally, lack of education and awareness regarding balanced nutrition and healthy lifestyle practices contributes to poor dietary decisions. Together, these environmental and socioeconomic influences significantly increase the risk of obesity in both developed and developing populations [19].

Medications

Certain medications can contribute to weight gain and increase the risk of obesity as a side effect. These drugs may alter appetite regulation, metabolism, fat storage, or energy expenditure in the body. Commonly implicated medications include corticosteroids, some antidepressants, antipsychotics, antiepileptic drugs, and insulin or other antidiabetic agents. Corticosteroids may increase appetite and promote fat redistribution, especially in the abdominal region. Antidepressants and antipsychotic drugs can affect neurotransmitters involved in appetite control, leading to increased food intake and reduced physical activity. Some antiepileptic medications may slow metabolism and promote weight gain over long-term use. Additionally, insulin therapy in diabetic patients can sometimes lead to increased fat storage due to improved glucose utilization. These medication-induced changes in metabolism and appetite can contribute significantly to gradual weight gain and the development or worsening of obesity in susceptible individuals [20-21].

Pathophysiology of Obesity

The pathophysiology of obesity is highly complex and involves dysregulation of neuroendocrine control of energy balance, abnormal adipose tissue function, metabolic alterations, and chronic low-grade inflammation. At the core of this process is the hypothalamus, which regulates appetite and satiety by integrating peripheral hormonal signals. Key hormones such as leptin, insulin, and ghrelin communicate the body's energy status to the brain. In normal physiology, leptin suppresses appetite and promotes energy expenditure; however, in obesity, leptin resistance develops, leading to impaired satiety signaling and persistent hyperphagia (increased food intake). Similarly, insulin resistance reduces glucose uptake in peripheral tissues and promotes lipogenesis, thereby enhancing fat storage.

Adipose tissue, once considered a passive energy storage site, is now recognized as an active endocrine organ. In obesity, adipocytes undergo hypertrophy and hyperplasia, leading to altered secretion of adipokines. There is an increased release of pro-inflammatory cytokines such as TNF-α, IL-6, and MCP-1, while anti-inflammatory adiponectin levels decrease. This shift promotes a state of chronic low-grade systemic inflammation, which is a key feature of obesity and contributes to the development of metabolic complications.

Excess caloric intake leads to increased triglyceride synthesis and storage in adipose tissue. When storage capacity is exceeded, lipids accumulate in non-

adipose tissues such as the liver, skeletal muscle, and pancreas, a condition known as ectopic fat deposition. This contributes to insulin resistance, hepatic steatosis, and impaired β-cell function. Mitochondrial dysfunction further aggravates the condition by reducing fatty acid oxidation and energy expenditure, while increasing reactive oxygen species (ROS) production, leading to oxidative stress.

Hormonal imbalances also play a crucial role in obesity pathophysiology. Increased ghrelin levels stimulate appetite, while reduced peptide YY and adiponectin levels impair satiety and metabolic regulation. Elevated cortisol levels, often due to chronic stress, promote visceral fat accumulation and worsen metabolic disturbances. These hormonal changes collectively disrupt normal energy homeostasis.

Over time, these interconnected mechanisms create a persistent positive energy balance, leading to progressive weight gain and expansion of adipose tissue mass. This condition ultimately predisposes individuals to serious metabolic disorders such as Type 2 Diabetes, Hypertension, dyslipidaemia, cardiovascular diseases, and non-alcoholic fatty liver disease. Thus, obesity is not merely an excess of body fat but a systemic metabolic disorder involving multiple physiological pathways [21-22].

MATERIALS AND METHODS

Collection of Plant Materials:

Dried Hygrophila auriculatawas purchased from an herbal Market of Hyderabad, Telangana, India, and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V University, and Tirupati.

Preparation of plant extract [23]

The fresh Leaf's was air dried in the shade and extracted with ethanolic extract, using a Soxhlet extractor for 8 hrs at 55-60^{0}C. The supernatant was filtered through Whatman filter paper No.1 and concentrated under reduced pressure using a vacuum at 44 ± 10^{0}C in a rotavapor (IKA ® RB 10 Rota Evaporator, India) The extract was stored at 22^{0}C in a seeded air-tight container.

The percentage of extract yield was calculated by using the formula

$$\%\ of\ extract\ yield = \frac{weight\ in\ gm\ of\ extract\ obtaine}{weight\ in\ gm\ of\ plant\ material\ ta}$$

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Experimental Animals

Healthy adult Wistar albino rats of either sex, weighing between 180 and 220 g, were used for the present study. The animals were procured from a CPCSEA-registered animal breeding facility and acclimatized to the laboratory conditions for one week before the commencement of the experiment. The rats were housed in clean polypropylene cages with sterile paddy husk bedding under standard laboratory conditions, maintained at a temperature of 22 ± 2°C, relative humidity of 55 ± 10%, and a 12 h light/12 h dark cycle. Animals had free access to a standard laboratory pellet diet and drinking water *ad libitum* during the acclimatization period. During the obesity induction phase, the experimental groups received a high-fat diet (HFD), while the normal control group continued on the standard pellet diet. All experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by the Institutional Animal Ethics Committee (IAEC) of the respective institution before the initiation of the study.

Obesity Induction by High-Fat Diet (HFD) [24]

Obesity was induced in Wistar albino rats by feeding them a high-fat diet (HFD) for 6–8 weeks. The rats in the experimental groups (Groups II–VI) received the HFD *ad libitum*, while the normal control group was maintained on a standard laboratory pellet diet. The high-fat diet was formulated to provide approximately 45–60% of total calories from fat, along with adequate amounts of carbohydrates, proteins, vitamins, and minerals. The HFD typically consisted of ingredients such as lard, casein, cholesterol, sucrose, corn starch, cellulose, vitamin and mineral mixtures, and coconut oil or soybean oil. During the induction period, body weight and food intake were recorded weekly to monitor the development of obesity. Obesity was confirmed by a significant increase in body weight, body mass

index (BMI), Lee index, and adiposity compared with the normal control group. Following successful induction of obesity, treatment with the standard drug (Orlistat) and the ethanolic extract of *Hygrophila auriculata* was administered orally once daily for 28 consecutive days, while the animals continued to receive the high-fat diet throughout the treatment period.

Animal Grouping

Group I Normal Control (Standard pellet diet + Normal saline) Vehicle (10 mL/kg, p.o.)

Group II Obese Control (High-Fat Diet)Vehicle (10 mL/kg, p.o.)

Group III Standard Drug + High-Fat DietOrlistat (10 mg/kg, p.o.)

Group IV Hygrophila auriculata Ethanolic Extract + High-Fat Diet100 mg/kg, p.o.

Group V Hygrophila auriculata Ethanolic Extract + High-Fat Diet200 mg/kg, p.o.

Group VI Hygrophila auriculata Ethanolic Extract + High-Fat Diet400 mg/kg, p.o.

Body Weight [25-26]

Body weight was measured to evaluate the progression of obesity and the anti-obesity effect of the ethanolic extract of *Hygrophila auriculata*. Each rat was weighed individually using a calibrated digital electronic balance before the commencement of the study to obtain the initial body weight. Thereafter, body weight was recorded at weekly intervals throughout the obesity induction period and the treatment period. The measurements were taken at the same time of day to minimize variations due to feeding and circadian rhythm. The final body weight was recorded at the end of the experimental period before sacrifice. Changes in body weight were calculated by subtracting the initial body weight from the final body weight and were expressed in grams (g). The percentage body weight gain was also determined to assess the effectiveness of the treatment.

Calculation:

Body Weight Gain (g) = Final Body Weight − Initial Body Weight

Percentage Body Weight Gain (%) = $\frac{\text{Final Body Weight} - \text{Initial Body Weight}}{\text{Initial Body Weight}} \times 100$

Body Mass Index (BMI) [27]

Body Mass Index (BMI) was determined to assess the degree of obesity in experimental rats. At the end of the experimental period, each rat was weighed using a calibrated digital weighing balance to obtain the body weight (g). The naso-anal length (NAL), defined as the distance from the tip of the nose to the anus, was measured in centimeters (cm) using a non-stretchable measuring tape while the animal was gently restrained in a straight position. BMI was then calculated using the following formula:

BMI (g/cm²) = Body weight (g) / [Naso-anal length (cm)]²

The BMI values were recorded for all animals and compared among the experimental groups. An increase in BMI indicates obesity, whereas a reduction in BMI following treatment reflects the anti-obesity effect of the test extract.

Formula:

$$\text{BMI}\,(\text{g/cm}^2) = \frac{\text{Body Weight (g)}}{(\text{Naso-anal Length (cm)})^2}$$

Lee Index [28-29]

The Lee Index was determined as an anthropometric indicator of obesity in experimental rats. At the end of the treatment period, the body weight of each rat was measured using a calibrated digital balance, and the naso-anal length (NAL) was measured in centimeters using a non-stretchable measuring tape. The Lee Index was calculated by taking the cube root of the body weight (g), dividing it by the naso-anal length (cm), and multiplying the result by 1000. An increase in the Lee Index indicates greater adiposity and obesity, whereas a decrease following treatment reflects the anti-obesity effect of the test extract.

Formula:

$$\text{Lee Index} = \left(\frac{\sqrt[3]{\text{Body Weight (g)}}}{\text{Naso-anal Length (cm)}} \right) \times 1000$$

where:

- Body weight is expressed in grams (g).
- Naso-anal length is expressed in centimeters (cm).

The Lee Index was calculated for all experimental animals and compared among the different treatment groups.

Estimation of Total Cholesterol (TC) [30-31]

Serum total cholesterol (TC) was estimated using a commercially available enzymatic colorimetric diagnostic kit based on the cholesterol oxidase–peroxidase (CHOD–POD) method. At the end of the experimental period, blood samples were collected by retro-orbital puncture or cardiac puncture under light anesthesia. The blood was allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes to separate the serum. For the assay, the working reagent was mixed with the serum sample according to the manufacturer's instructions and incubated at 37°C for 10 minutes (or at room temperature for 15 minutes). During the reaction, cholesterol esters were hydrolyzed by cholesterol esterase to free cholesterol, which was subsequently oxidized by cholesterol oxidase to produce hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacted with chromogenic substrates to form a colored quinoneimine complex. The absorbance was measured at 500–520 nm using a UV–Visible spectrophotometer against a reagent blank. The concentration of total cholesterol was calculated using a cholesterol standard and expressed as mg/dL.

Calculation:

$$\text{Total Cholesterol (mg/dL)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}}$$

Estimation of Triglycerides (TG) [32-33]

Serum triglyceride (TG) levels were estimated using a commercially available enzymatic colorimetric diagnostic kit based on the glycerol-3-phosphate oxidase–peroxidase (GPO–POD) method. At the end of the experimental period, blood samples were collected under light anesthesia, and serum was separated by centrifugation at 3000 rpm for 10 minutes. For the assay, the serum sample was mixed with the working reagent according to the manufacturer's protocol and incubated at 37°C for 10 minutes (or at room temperature for 15 minutes). During the reaction, triglycerides were hydrolyzed by lipase to glycerol and free fatty acids. The glycerol was phosphorylated by glycerol kinase and subsequently oxidized by glycerol-3-phosphate oxidase to produce hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacted with chromogenic substrates to form a colored quinoneimine complex. The absorbance was measured at 500–520 nm using a UV–Visible spectrophotometer against a reagent blank. The triglyceride concentration was calculated using a standard solution and expressed as mg/dL.

Calculation:

$$\text{Triglycerides (mg/dL)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Concentrat}$$

Estimation of High-Density Lipoprotein Cholesterol (HDL-C) [34-35]

HDL-cholesterol (HDL-C) was estimated using a commercially available enzymatic colorimetric diagnostic kit based on the cholesterol oxidase–peroxidase (CHOD–POD) method after selective precipitation of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL). At the end of the experimental period, blood samples were collected under light anesthesia, and serum was separated by centrifugation at 3000 rpm for 10 minutes. The serum was treated with a precipitating reagent containing phosphotungstic acid and magnesium chloride (or an equivalent reagent) to precipitate LDL and VLDL. The mixture was centrifuged, and the clear supernatant containing HDL-cholesterol was collected. The supernatant was then mixed with the HDL working reagent and incubated at 37°C for 10 minutes (or at room temperature for 15 minutes). The absorbance of the resulting colored complex was measured at 500–520 nm using a UV–Visible spectrophotometer against a reagent blank. The HDL-cholesterol concentration was calculated using an HDL cholesterol standard and expressed as mg/dL.

Calculation:

HDL-Cholesterol (mg/dL) = (Absorbance of Sample / Absorbance of Standard) × Concentration of Standard

Higher HDL-cholesterol levels are indicative of improved lipid metabolism and a protective effect against obesity-associated dyslipidaemia.

Estimation of Low-Density Lipoprotein Cholesterol (LDL-C) [36]

Low-density lipoprotein cholesterol (LDL-C) was estimated from the serum lipid profile using the Friedwald equation, after determining serum total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) by enzymatic colorimetric methods. At the end of the experimental period, blood samples were collected under light anaesthesia, and serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum total cholesterol, triglycerides, and HDL-cholesterol were analyzed using commercially available enzymatic diagnostic kits. The LDL-cholesterol concentration was then calculated using the Friedwald formula and expressed as mg/dL.

Calculation:

LDL-Cholesterol (mg/dL) = Total Cholesterol − HDL-Cholesterol − (Triglycerides / 5)

Estimation of Very Low-Density Lipoprotein Cholesterol (VLDL-C) [37]

Very low-density lipoprotein cholesterol (VLDL-C) was estimated indirectly from the serum triglyceride (TG) concentration using the Friedewald equation. At the end of the experimental period, blood samples were collected under light anaesthesia, and serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum triglyceride levels were determined using a commercially available enzymatic colorimetric kit based on the glycerol-3-phosphate oxidase–peroxidase (GPO–POD) method. The VLDL-cholesterol concentration was then calculated by dividing the serum triglyceride value by 5 and expressed as mg/dL.

Calculation:

VLDL-Cholesterol (mg/dL) = Triglycerides (mg/dL) / 5

Estimation of Malondialdehyde (MDA) [38]

Malondialdehyde (MDA), a marker of lipid peroxidation, was estimated by the thiobarbituric acid reactive substances (TBARS) method. Liver tissue (or adipose tissue) was homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4) to prepare a 10% (w/v) homogenate, which was centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was used for the assay. An aliquot of the homogenate was mixed with 0.8% thiobarbituric acid (TBA), 20% acetic acid (pH 3.5), and 8.1% sodium dodecyl sulfate (SDS). The reaction mixture was heated in a boiling water bath (95–100°C) for 60 minutes, cooled, and centrifuged. The absorbance of the pink chromogen was measured at 532 nm using a UV–Visible spectrophotometer. The MDA concentration was expressed as nmol of MDA/mg protein.

Estimation of Superoxide Dismutase (SOD) [39]

Superoxide dismutase (SOD) activity was estimated by the method of Misra and Fridovich (1972) based on the inhibition of epinephrine auto-oxidation. A 10% tissue homogenate was prepared in ice-cold phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 minutes at 4°C. The reaction mixture containing carbonate buffer (pH 10.2), EDTA, and tissue supernatant was prepared, and the reaction was initiated by adding freshly prepared epinephrine solution. The increase in absorbance due to adrenochrome formation was measured at 480 nm for 3 minutes using a UV–Visible spectrophotometer. One unit of SOD activity was defined as the amount of enzyme required to inhibit epinephrine auto-oxidation by 50%, and the results were expressed as U/mg protein.

Estimation of Catalase (CAT) [40]

Catalase (CAT) activity was determined according to the method of Aebi (1984) by measuring the decomposition of hydrogen peroxide (H_2O_2). A 10% tissue homogenate was prepared in ice-cold phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 minutes at 4°C. The reaction mixture consisted of phosphate buffer, hydrogen peroxide solution, and tissue supernatant. The decrease in absorbance due to decomposition of hydrogen peroxide was recorded at 240 nm for 3 minutes using a UV–Visible spectrophotometer. Catalase activity was expressed as U/mg protein.

4. Estimation of Reduced Glutathione (GSH)

Reduced glutathione (GSH) was estimated by the method of Ellman (1959) using 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). A 10% tissue homogenate was prepared in ice-cold phosphate buffer and centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was mixed with 10% trichloroacetic acid (TCA) to precipitate proteins and centrifuged again.

The clear supernatant was then mixed with phosphate buffer and DTNB reagent. The yellow-colored chromogen formed due to the reaction between DTNB and sulfhydryl groups was measured at 412 nm using a UV –Visible spectrophotometer. The GSH concentration was calculated from a standard curve and expressed as µmol/mg protein.

Statistical evaluation

All values were represented as mean values ± SEM (standard error of the mean), and the data was analyzed using one way ANOVA followed by a Dunnett's t-test using GraphPad Prism 9. The results were considered statistically significant if $p < 0.05$, $p < 0.01$, or $p < 0.001$.

RESULTS

Percentage of yield

$$Percentage\ of\ yield = \frac{Weight\ of\ crude\ extract}{Weight\ of\ dried\ material} \times 100$$

$$Percentage\ of\ yield = \frac{18\,g}{50\,g} \times 100 = 36\ \%$$

Phytochemical Test Results

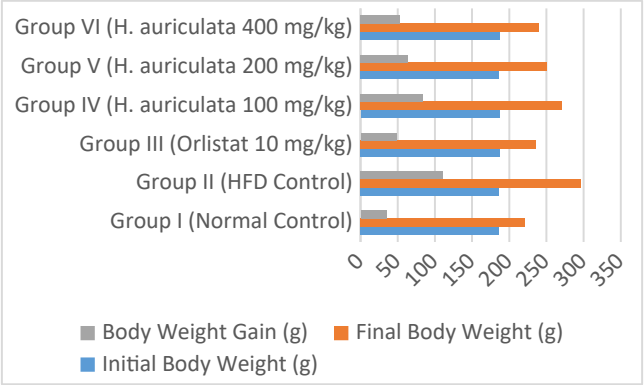
S. No.	Phytochemical Constituent	Result
1	Alkaloids	+
2	Alkaloids	+
3	Carbohydrates	+
4	Reducing Sugars	+
5	Glycosides	+
6	Flavonoids	+
7	Phenolic Compounds	+
8	Tannins	+
9	Saponins	+
10	Steroids	+
11	Terpenoids	+
12	Proteins	+
13	Amino Acids	+
14	Fixed Oils and Fats	+

Effect of Ethanolic Extract of *Hygrophila auriculata* on Body Weight in High-Fat Diet-Induced Obese Rats

Group	Initial Body Weight (g)	Final Body Weight (g)	Body Weight Gain (g)
Group I (Normal Control)	186.42 ± 2.81	221.35 ± 3.24	34.93 ± 1.42
Group II (HFD Control)	185.96 ± 2.63	296.84 ± 4.56***	110.88 ± 3.12***
Group III (Orlistat 10 mg/kg)	187.24 ± 2.75	235.62 ± 3.48###	48.38 ± 2.06###
Group IV (H. auriculata 100 mg/kg)	186.87 ± 2.58	270.41 ± 4.21*	83.54 ± 2.84*
Group V (H. auriculata 200 mg/kg)	186.51 ± 2.71	250.82 ± 3.75##	64.31 ± 2.33##
Group VI (H. auriculata 400 mg/kg)	187.03 ± 2.69	239.76 ± 3.39###	52.73 ± 2.15###

Administration of a high-fat diet for the experimental period resulted in a significant increase in body weight in the obese control group (Group II) compared with the normal control group ($P < 0.001$), confirming successful induction of obesity. Treatment with the standard drug, Orlistat (10 mg/kg), markedly reduced body weight gain compared with the obese control ($P < 0.001$). Similarly, the ethanolic extract of Hygrophila auriculata produced a dose-dependent reduction in body weight gain. Rats treated with 100 mg/kg showed a modest but significant decrease ($P < 0.05$), whereas the 200 mg/kg and 400 mg/kg doses produced a greater reduction ($P < 0.01$ and $P < 0.001$, respectively). The highest dose (400 mg/kg) demonstrated body weight

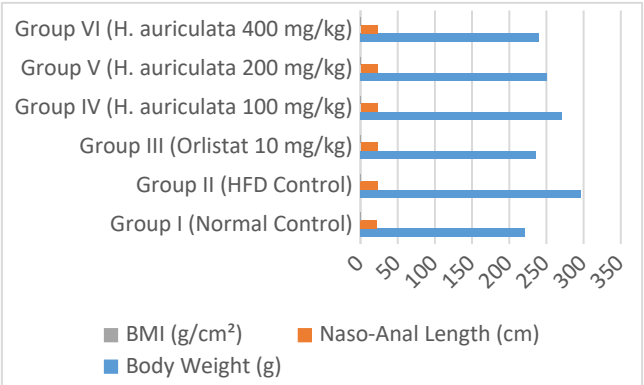
values comparable to those of the standard treatment, suggesting a significant anti-obesity effect of the extract in high-fat diet-induced obese rats.



Effect of Ethanolic Extract of *Hygrophila auriculata* on Body Mass Index (BMI) in High-Fat Diet-Induced Obese Rats

Group	Body Weight (g)	Naso-Anal Length (cm)	BMI (g/cm²)
Group I (Normal Control)	221.35 ± 3.24	22.60 ± 0.18	0.433 ± 0.008
Group II (HFD Control)	296.84 ± 4.56***	22.92 ± 0.16	0.565 ± 0.010***
Group III (Orlistat 10 mg/kg)	235.62 ± 3.48###	22.74 ± 0.17	0.456 ± 0.009###
Group IV (H. auriculata 100 mg/kg)	270.41 ± 4.21*	22.83 ± 0.15	0.519 ± 0.011*
Group V (H. auriculata 200 mg/kg)	250.82 ± 3.75##	22.79 ± 0.18	0.483 ± 0.009##
Group VI (H. auriculata 400 mg/kg)	239.76 ± 3.39###	22.75 ± 0.16	0.463 ± 0.008###

The Body Mass Index (BMI) was significantly increased in the high-fat diet (HFD) control group (Group II) compared with the normal control group ($P < 0.001$), indicating successful induction of obesity. Treatment with Orlistat (10 mg/kg) significantly reduced the BMI compared with the obese control group ($P < 0.001$). Similarly, administration of the ethanolic extract of *Hygrophila auriculata* produced a dose-dependent reduction in BMI. The 100 mg/kg dose showed a significant decrease ($P < 0.05$), while the 200 mg/kg and 400 mg/kg doses produced a more pronounced reduction ($P < 0.01$ and $P < 0.001$, respectively). The highest dose (400 mg/kg) reduced BMI values close to those observed in the Orlistat-treated group, indicating a marked anti-obesity effect of the extract through the reduction of body mass accumulation in HFD-induced obese rats.

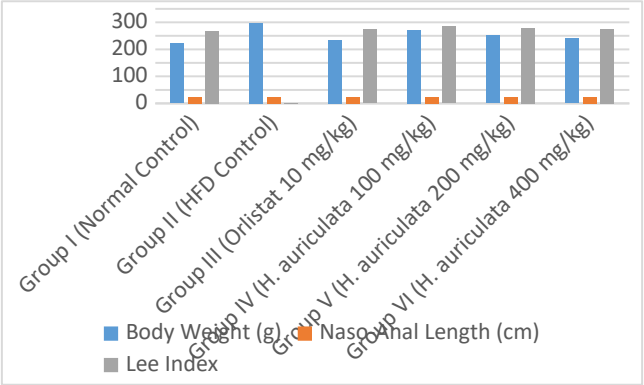


Effect of Ethanolic Extract of *Hygrophila auriculata* on Lee Index in High-Fat Diet-Induced Obese Rats

Group	Body Weight (g)	Naso-Anal Length (cm)	Lee Index
Group I (Normal Control)	221.35 ± 3.24	22.60 ± 0.18	267.84 ± 2.15

Group	Body Weight (g)	Naso-Anal Length (cm)	Lee Index
Group II (HFD Control)	296.84 ± 4.56***	22.92 ± 0.16	291.73 ± 2.48***
Group III (Orlistat 10 mg/kg)	235.62 ± 3.48###	22.74 ± 0.17	272.94 ± 2.06###
Group IV (H. auriculata 100 mg/kg)	270.41 ± 4.21*	22.83 ± 0.15	284.62 ± 2.31*
Group V (H. auriculata 200 mg/kg)	250.82 ± 3.75##	22.79 ± 0.18	278.11 ± 2.19##
Group VI (H. auriculata 400 mg/kg)	239.76 ± 3.39###	22.75 ± 0.16	274.56 ± 2.12###

The Lee Index was significantly increased in the high-fat diet (HFD) control group (Group II) compared with the normal control group ($P < 0.001$), indicating increased adiposity and successful induction of obesity. Treatment with the standard drug, Orlistat (10 mg/kg), significantly reduced the Lee Index ($P < 0.001$) compared with the obese control group. Administration of the ethanolic extract of *Hygrophila auriculata* also produced a dose-dependent reduction in the Lee Index. The low-dose group (100 mg/kg) showed a significant decrease ($P < 0.05$), whereas the medium-dose (200 mg/kg) and high-dose (400 mg/kg) groups exhibited greater reductions ($P < 0.01$ and $P < 0.001$, respectively). The highest dose demonstrated Lee Index values approaching those of the Orlistat-treated group, suggesting that the extract effectively reduced adiposity and improved obesity-related anthropometric parameters in high-fat diet-induced obese rats.

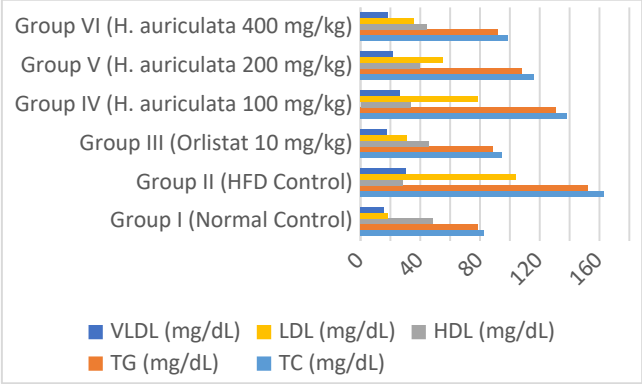


Extract of *Hygrophila auriculata* on Serum Lipid Profile in High-Fat Diet-Induced Obese Rats

Group	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Group I (Normal Control)	82.54 ± 2.14	78.36 ± 2.06	48.72 ± 1.42	18.15 ± 1.08	15.67 ± 0.41
Group II (HFD Control)	162.83 ± 3.68***	152.41 ± 3.21***	28.46 ± 1.18***	103.89 ± 2.84***	30.48 ± 0.64***
Group III (Orlistat 10 mg/kg)	94.72 ± 2.31###	88.64 ± 2.12###	45.86 ± 1.37###	31.13 ± 1.56###	17.73 ± 0.42###
Group IV (H. auriculata 100 mg/kg)	138.52 ± 3.24*	130.84 ± 2.86*	33.54 ± 1.24*	78.81 ± 2.37*	26.17 ± 0.57*
Group V (H. auriculata 200 mg/kg)	116.34 ± 2.78##	108.26 ± 2.54##	39.68 ± 1.29##	55.01 ± 1.94##	21.65 ± 0.51##
Group VI (H. auriculata 400 mg/kg)	98.46 ± 2.35###	92.18 ± 2.18###	44.32 ± 1.35###	35.70 ± 1.62###	18.44 ± 0.44###

The high-fat diet (HFD) control group exhibited a significant increase in serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels, along with a significant decrease in high-density lipoprotein (HDL) levels compared with the normal control group ($P < 0.001$), indicating the development of obesity-associated dyslipidaemia. Treatment with the standard drug, Orlistat (10 mg/kg), significantly restored the lipid profile toward normal values ($P < 0.001$). Administration of the ethanolic extract of *Hygrophila auriculata* also produced a dose-dependent

improvement in the serum lipid profile. The extract significantly reduced TC, TG, LDL, and VLDL levels while increasing HDL levels compared with the HFD control group. The 400 mg/kg dose showed the greatest improvement ($P < 0.001$), with lipid profile values approaching those observed in the Orlistat-treated group. These findings suggest that the ethanolic extract of *Hygrophila auriculata* possesses significant antihyperlipidemic and anti-obesity activity in high-fat diet-induced obese rats.



Effect of Ethanolic Extract of *Hygrophila auriculata* on Oxidative Stress Parameters in High-Fat Diet-Induced Obese Rats

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/mg protein)
Group I (Normal Control)	2.38 ± 0.12	14.86 ± 0.41	58.74 ± 1.26	8.92 ± 0.28
Group II (HFD Control)	5.96 ± 0.21***	7.18 ± 0.32***	31.82 ± 1.04***	4.16 ± 0.19***
Group III (Orlistat 10 mg/kg)	2.84 ± 0.14###	13.72 ± 0.38###	55.68 ± 1.18###	8.24 ± 0.25###
Group IV (H. auriculata 100 mg/kg)	4.62 ± 0.18*	9.54 ± 0.35*	40.76 ± 1.12*	5.72 ± 0.22*
Group V (H. auriculata 200 mg/kg)	3.56 ± 0.16##	11.84 ± 0.37##	48.42 ± 1.15##	7.02 ± 0.24##
Group VI (H. auriculata 400 mg/kg)	2.98 ± 0.13###	13.28 ± 0.40###	54.12 ± 1.21###	8.06 ± 0.27###

The high-fat diet (HFD) control group showed a significant increase in malondialdehyde (MDA) levels and a significant decrease in the activities of superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) compared with the normal control group ($P < 0.001$), indicating enhanced lipid peroxidation and oxidative stress. Treatment with the standard drug, Orlistat (10 mg/kg), markedly reduced MDA levels while restoring SOD, CAT, and GSH activities toward normal values ($P < 0.001$). Similarly, treatment with the ethanolic extract of *Hygrophila auriculata* produced a dose-dependent improvement in all oxidative stress parameters. The 100 mg/kg dose showed moderate antioxidant activity ($P < 0.05$), whereas the 200 mg/kg and 400 mg/kg doses significantly decreased MDA levels and enhanced endogenous antioxidant enzyme activities ($P < 0.01$ and $P < 0.001$, respectively). The highest dose (400 mg/kg) demonstrated antioxidant effects comparable to those of Orlistat, suggesting that the extract effectively protects against obesity-induced oxidative damage through its potent antioxidant properties.

DISCUSSION

The present study was undertaken to evaluate the anti-obesity potential of the ethanolic extract of *Hygrophila auriculata* in high-fat diet (HFD)-induced obese rats. Obesity induced by prolonged consumption of a high-fat diet resulted in significant increases in body weight, body mass index (BMI), Lee index, and serum lipid parameters, along with marked oxidative stress, as evidenced by elevated malondialdehyde (MDA) levels and reduced antioxidant enzyme activities. Treatment with the ethanolic extract of *Hygrophila auriculata* significantly ameliorated these alterations in a dose-dependent manner, indicating its protective effect against obesity and its associated metabolic disturbances.The HFD-fed rats exhibited a marked increase in body weight compared with the normal control group, confirming the successful induction of obesity. Excess dietary fat promotes adipocyte hypertrophy and hyperplasia, leading to excessive fat accumulation and increased body mass. Administration of the ethanolic extract significantly reduced body weight gain, BMI, and Lee index, particularly at doses of 200 and 400 mg/kg. The reduction in these anthropometric parameters suggests that the extract suppresses fat accumulation and improves overall body composition. These effects may be attributed to the presence of flavonoids, phenolic compounds, saponins, and phytosterols, which have been reported to regulate lipid metabolism, inhibit adipogenesis, and improve energy expenditure.

Obesity is commonly associated with dyslipidaemia characterized by elevated serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), together with reduced high-density lipoprotein (HDL). In the present study, HFD-fed rats showed significant deterioration of the lipid profile compared with the normal control group. Treatment with *Hygrophila auriculata* significantly lowered TC, TG, LDL, and VLDL levels while increasing HDL levels in a dose-dependent manner. These findings indicate that the extract possesses potent antihyperlipidemic activity, possibly by reducing intestinal lipid absorption, enhancing lipid catabolism, suppressing hepatic cholesterol synthesis, and promoting reverse cholesterol transport. The lipid-lowering effect observed at the highest dose was comparable to that of the standard drug, Orlistat.Oxidative stress is a major pathological mechanism involved in obesity and its metabolic complications. Excessive fat accumulation increases the production of reactive oxygen species (ROS), resulting in lipid peroxidation, mitochondrial dysfunction, and cellular injury. In the present investigation, HFD significantly increased MDA levels while reducing the activities of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). Treatment with the ethanolic extract markedly reduced MDA levels and restored antioxidant enzyme activities, demonstrating its strong antioxidant potential. These beneficial effects are likely attributable to the abundant flavonoids and phenolic compounds present in *Hygrophila auriculata*, which scavenge free radicals, inhibit lipid peroxidation, and enhance endogenous antioxidant defense mechanisms.

The observed pharmacological effects of *Hygrophila auriculata* may be explained by its rich phytochemical composition, including flavonoids such as quercetin, apigenin, and luteolin, along with phenolic compounds, saponins, terpenoids, lupeol, β-sitosterol, and stigmasterol. These bioactive constituents have been reported to possess antioxidant, anti-inflammatory,

antihyperlipidemic, and anti-obesity activities. Flavonoids improve lipid metabolism by activating AMP-activated protein kinase (AMPK), inhibiting adipocyte differentiation, and promoting fatty acid oxidation. Phytosterols reduce intestinal cholesterol absorption, whereas saponins enhance bile acid excretion and decrease lipid absorption. The combined action of these phytoconstituents may account for the significant anti-obesity effects observed in the present study.

Overall, the results demonstrate that the ethanolic extract of *Hygrophila auriculata* effectively attenuated obesity-induced metabolic alterations by reducing body weight gain, improving anthropometric indices, normalizing the serum lipid profile, and enhancing antioxidant defense systems. The extract exhibited dose-dependent efficacy, with the 400 mg/kg dose producing effects comparable to the standard drug, Orlistat. These findings provide scientific evidence supporting the traditional use of *Hygrophila auriculata* in the management of obesity and suggest that it may serve as a promising natural therapeutic agent for the prevention and treatment of obesity and related metabolic disorders. Further studies are warranted to isolate the active constituents, elucidate the underlying molecular mechanisms, and confirm its long-term safety and efficacy through clinical investigations.

CONCLUSION

The present study demonstrated that the ethanolic extract of *Hygrophila auriculata* possesses significant anti-obesity activity in high-fat diet-induced obese rats. Administration of the extract produced a dose-dependent reduction in body weight gain, body mass index (BMI), and Lee index, indicating its effectiveness in controlling obesity-associated anthropometric changes. The extract also significantly improved the serum lipid profile by decreasing total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels while increasing high-density lipoprotein (HDL) levels, thereby ameliorating obesity-induced dyslipidemia.Furthermore, treatment with the ethanolic extract markedly reduced lipid peroxidation, as evidenced by decreased malondialdehyde (MDA) levels, and enhanced the activities of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). These findings indicate that the extract exerts a protective effect against oxidative stress associated with obesity. The observed pharmacological effects are likely attributable to the presence of bioactive phytoconstituents such as flavonoids, phenolic compounds, saponins, terpenoids, and phytosterols, which possess antioxidant, antihyperlipidemic, and metabolic regulatory properties.

Among the tested doses, the 400 mg/kg ethanolic extract of *Hygrophila auriculata* exhibited the most pronounced anti-obesity activity and produced results comparable to those of the standard drug, Orlistat (10 mg/kg). Overall, the findings provide scientific evidence supporting the traditional use of *Hygrophila auriculata* as a natural therapeutic agent for the management of obesity and its associated metabolic complications. Further phytochemical, pharmacological, and clinical investigations are recommended to identify the active constituents, elucidate their molecular mechanisms of action, and establish the long-term safety and therapeutic efficacy of the plant in the treatment of obesity.

REFERENCES

1. Bray GA, Frühbeck G, Ryan DH, Wilding JPH. Management of obesity. *Lancet*. 2016;387 (10031):1947-1956. doi:10.1016/S0140-6736 (16)00271-3.
2. Blüher M. Obesity: global epidemiology and pathogenesis. *Nat Rev Endocrinol*. 2019;15 (5):288-298. doi:10.1038/s41574-019-0176-8.
3. Heymsfield SB, Wadden TA. Mechanisms, pathophysiology, and management of obesity. *N Engl J Med*. 2017;376 (3):254-266. doi:10.1056/NEJMra1514009.
4. Hruby A, Hu FB. The epidemiology of obesity: A big picture. *Pharmacoeconomics*. 2015;33 (7):673-689. doi:10.1007/s40273-014-0243-x.
5. Ng M, Fleming T, Robinson M, et al. Global, regional, and national prevalence of overweight and obesity. *Lancet*. 2014;384 (9945):766-781. doi:10.1016/S0140-6736 (14)60460-8.

6. Frühbeck G, Busetto L, Dicker D, et al. The ABCD of obesity. *Obes Facts*. 2019;12 (2):154-164. doi:10.1159/000497124.
7. Apovian CM. Obesity: definition, comorbidities, causes, and burden. *Am J Manag Care*. 2016;22 (7 Suppl):s176-s185.
8. Müller TD, Blüher M, Tschöp MH, DiMarchi RD. Anti-obesity drug discovery: advances and challenges. *Nat Rev Drug Discov*. 2022;21 (3):201-223. doi:10.1038/s41573-021-00337-8.
9. Wilding JPH. Medication use for the treatment of diabetes and obesity. *Endocr Rev*. 2022;43 (4):639-661.
10. Drucker DJ. Mechanisms of action and therapeutic application of GLP-1. *Cell Metab*. 2018;27 (4):740-756. doi:10.1016/j.cmet.2018.03.001.
11. Astrup A, Carraro R, Finer N, et al. Safety, tolerability and sustained weight loss with liraglutide. *Int J Obes*. 2012;36 (6):843-854.
12. Pi-Sunyer X, Astrup A, Fujioka K, et al. A randomized trial of liraglutide for weight management. *N Engl J Med*. 2015;373 (1):11-22.
13. Wilding JPH, Batterham RL, Calanna S, et al. Once-weekly semaglutide in adults with overweight or obesity. *N Engl J Med*. 2021;384 (11):989-1002.
14. Garvey WT, Mechanick JI, Brett EM, et al. American Association of Clinical Endocrinologists clinical practice guidelines for obesity. *EndocrPract*. 2016;22 (Suppl 3):1-203.
15. Greenway FL. Physiological adaptations to weight loss and obesity treatment. *Obesity*. 2015;23 (6):1093-1099.
16. Rosen ED, Spiegelman BM. What we talk about when we talk about fat. *Cell*. 2014;156 (1-2):20-44.
17. Friedman JM. Leptin and regulation of body weight. *Nature*. 1998;395 (6704):763-770.
18. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature*. 2000;404 (6778):661-671.
19. Morton GJ, Meek TH, Schwartz MW. Neurobiology of food intake. *Nat Rev Neurosci*. 2014;15 (6):367-378.
20. Kahn BB, Flier JS. Obesity and insulin resistance. *J Clin Invest*. 2000;106 (4):473-481.
21. Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006;444 (7121):860-867.
22. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest*. 2017;127 (1):1-4.
23. Bays HE, González-Campoy JM, Bray GA, et al. Pathogenic potential of adipose tissue. *Endocr-Pract*. 2008;14 (8):977-990.
24. Torgerson JS, Hauptman J, Boldrin MN, Sjöström L. Xenical in the prevention of diabetes in obese subjects (XENDOS). *Diabetes Care*. 2004;27 (1):155-161.
25. Sjöström L. Review of bariatric surgery outcomes. *N Engl J Med*. 2013;369 (9):879-881.
26. Novelli ELB, Diniz YS, Galhardi CM, et al. Anthropometrical parameters and markers of obesity in rats. *Laboratory Animals.* 2007;41 (1):111–119.
27. Levin BE, Dunn-Meynell AA. Defense of body weight depends on dietary composition and palatability in rats with diet-induced obesity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology.* 2002;282:R46–R54.
28. Bernardis LL, Patterson BD. Correlation between Lee index and carcass fat content in weanling and adult female rats with hypothalamic lesions. *Journal of Endocrinology.* 1968;40 (4):527–528.
29. Levin BE, Dunn-Meynell AA. Defense of body weight depends on dietary composition and palatability in rats with diet-induced obesity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology.* 2002;282:R46–R54.
30. Tietz NW. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.* 6th ed. Elsevier; 2018.
31. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. *Clinical Chemistry.* 1973;19 (5):476–482.

32. Tietz NW. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.* 6th ed. Elsevier; 2018.
33. Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *Journal of Lipid Research.* 1970;11 (6):583–595.
34. Tietz NW. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.* 6th ed. Elsevier; 2018.
35. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry.* 1972;18 (6):499–502.
36. Tietz NW. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.* 6th ed. Elsevier; 2018.
37. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;95 (2):351–358.
38. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem.* 1972;247 (10):3170–3175.
39. Aebi H. Catalase in vitro. *Methods Enzymol.* 1984;105:121–126.
40. Ellman GL. Tissue sulfhydryl groups. *Arch BiochemBiophys.* 1959;82 (1):70–77.

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