STUDY OF ANTIOBESITY POTENTIAL OF "*Aeginetia Indica*" IN HFD-INDUCED OBESE RATS: A Review

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Abstract*— Aeginetia Indica is an underutilized bioactive compound containing flavonoids, phenolic acids and alkaloids which are popular for their anti-inflammatory properties. The plant is rich in antioxidants which can help in counteracting oxidative stress caused by high-fat diet (HFD), reducing risk of obesity-related complications. This study explores the antiobesity potential of Aeginetia Indica in a high-fat diet (HFD) induced obesity model using Sprague Dawley rats. Total 30 Rats were weighed first and then randomly allocated in to 5 groups having 6 rats in each group. Group 1 (Negative Control) Rats which are provided with oral normal diet and 0.9% normal saline throughout the study. Group 2 High fat rich diet control includes animals which were given high fat diet for 8 weeks. Group 3 (High fat diet + Test Drug in low dose) is treatment group which is given with low dose of out plant extract which we have obtained from plant *Aeginetia Indica* once a day for 42 days. Group 4 (High fat diet + Test Drug high dose) is treatment group which is given with high dose of out plant extract which we have obtained from plant *Aeginetia Indica* once a day for 42 days. Group 5 (High fat diet + Standard Orlistat 30 mg/kg) it include the standard group which is given the standard drug Orlistat 30mg per kg body weight once a day. All groups are fed with the high fat rich diet except normal control and their weight is measured weekly. Treatment impact of AI on body weight, liver function parameters and histopathological evaluations of liver and adipose tissue were investigated. The study on the Antiobesity potential of "*Aeginetia Indica*" in HFD-induced obese rats is expected to conclude that this novel formulation is a safe, effective and patient-friendly alternative to control the obesity.

***Index Terms*— Bioactive, Anti-Inflammatory, Histopathological, Formulation.**

I. INTRODUCTION

The human body's composition can be categorized into fat-free mass and body fat. Fat-free mass includes all non-fat components like bones, water, muscles, teeth, and connective tissues. Body fat consists of essential fats, vital for functions such as warmth and metabolic processes (making up 3% of body weight in men and 12% in women), and non-essential fat, stored in adipose tissue beneath the skin and around organs. An accumulation of this non-essential fat, resulting from a prolonged energy imbalance where calorie intake surpasses expenditure, leads to overweight and obesity. Body mass index (BMI), a straightforward measure of weight relative to height (kg/m²), is commonly used to classify these conditions in adults. Obesity can be experimentally induced through various methods, including diet-induced obesity, hypothalamic obesity, high-fat diets, gold-thio-glucose injection, monosodium glutamate (MSG) administration and the use of spontaneously obese rats.

Table 1 WHO Classification of Weight Status

WHO CLASSIFICATION OF WEIGHT STATUS	
WEIGHT STATUS	**BODY MASS INDEX (BMI), kg/m²**
Underweight	<18.5
Normal range	18.5-24.9
Overweight	25.0-29.9
Obese	≥30
Obese class I	30.0-34.9
Obese class II	35.0-39.9
Obese class III	≥40

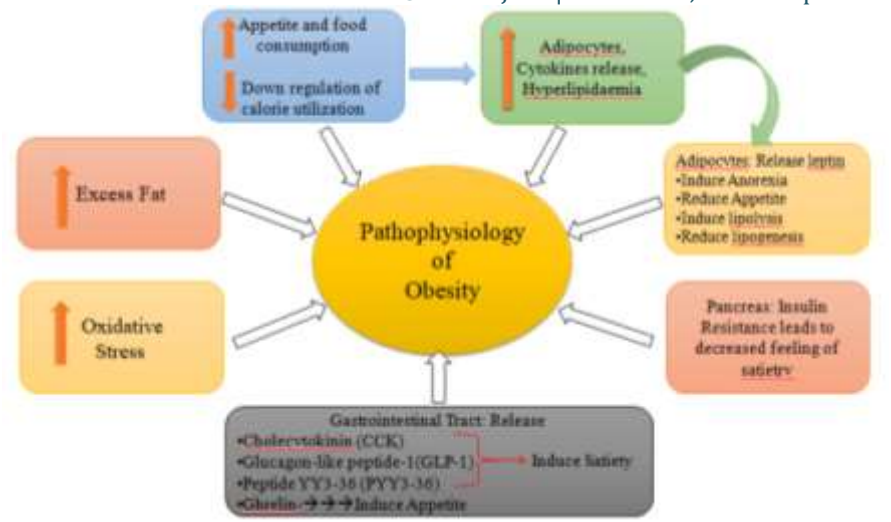

Figure 1 Pathophysiology of Obesity

II. FACTORS INFLUENCING OBESITY

- **Age:**
  - Obesity during childhood is recognized as a significant predictor of obesity in adulthood.
  - Body fat composition naturally tends to increase throughout adulthood, with the highest rates of overweight and obesity typically observed between the ages of 55 and 65.
- **Sex:**
  - Generally, women tend to have a higher proportion of body fat compared to men.
  - The prevalence of obesity can vary significantly between sexes depending on the specific population or ethnic group being studied.
- **Socioeconomic Status (SES):**
  - The relationship between SES and obesity prevalence can differ depending on the economic context of a country. In less affluent nations, higher SES may be associated with a greater prevalence of obesity. Conversely, in wealthier nations, lower SES groups may experience higher rates of obesity.
- **Energy Intake:**
  - Consistently consuming more calories than the body expends (overfeeding) leads to weight gain and can ultimately result in obesity.
- **Dietary Fat Intake:**
  - Ecological studies have indicated a correlation between the amount of fat consumed in the diet and the prevalence of overweight within populations.

Aeginetia Indica (Orobanchaceae) is a new genus record for Himachal Pradesh's flora. The plant was discovered growing on the roots of *Dendrocalamus strictus* in the Malan region of District Kangra. Traditional Thai desserts use *Aeginetia indica*, as a culinary coloring. It is indigenous to South and Southeast Asia. The plant, also known as forest ghost flower or Indian broomrape, has no leaves and its blossoms emerge abruptly from the ground. *Aeginetia Indica* is an annual herb. AI is a holoparasite. It is found in shaded forest floors which are covered with leaves litter. It has a slender fleshy fibrous root. Leaves are not present in it. Flower is solitary on a slender erect, of crimson purple colour corolla is tubular, curved. Host species are Zea mays Oryza sativa and Saccharum officinarum. It is 15-20 cm tall.

Figure 2 Aeginetia Indica

III. OBJECTIVE OF THE PRESENT WORK

In this study, we aim to investigate the efficacy of Aeginetia Indica as a natural inhibitor and its broader implications for antiobesity therapy. The study employed an HFD-induced obesity model on Sprague Dawley rats to explore the potential antiobesity effect of the ethanolic fruit extract of Aeginetia Indica.

IV. LITERATURE REVIEW

Foxcroft and Milne (2000) [01] clarified the potential benefits, disbenefits and costs of Orlistat for the effective treatment of the obesity. On average, Orlistat resulted in losing an additional 3-4% of the initial weight of the body in obese people over diet alone

during a two year period. There were no strong evidences that this short-term weight loss would have a longer-term impact on morbidity and mortality. The cost utility of Orlistat treatment was estimated at around 46,000 Pounds/Qty Adjusted Life Year gained (extreme values sensitivity analysis 14,000 Pounds to 132,000 Pounds). This rapid review highlighted important questions on the potential value of Orlistat for controlling the obesity. Further research was done to uncover the longer-term impact on mortality and morbidity from short-term weight loss.

Meara et.al. (2001) [02] worked to systematically assess the clinical effectiveness and cost-effectiveness of orlistat in management of the obesity. Randomised controlled trials (RCTs) evaluating the effectiveness of orlistat which is used for controlling the weight loss or maintenance of weight loss in overweight or obese patients were eligible for inclusion. Primary outcomes were deflections in body weight, fat content or fat distribution. Secondary outcomes were deflections in obesity-related risk-factor profiles, such as lipid levels, indicators of glycaemic control & blood pressure. Majority of the trials showed greater loss in the weight & maintenance of the better weight with the use of orlistat compared to placebo at all endpoints (statistically significant differences for both outcomes). Orlistat (120 mg) daily dosage of three times was optimum regimen in the terms of the weight loss. Orlistat demonstrated a positive impact on at least some lipid concentration parameters. Three randomized controlled trials (RCTs) showed that orlistat led to statistically significant decreases in blood pressure compared to a placebo. This indicates a potential benefit for cardiovascular health beyond just lipid management.

Diemen et al. (2006) [03] concluded that studying the causes and effects of obesity in animal models can lead to a deeper understanding of how obesity develops and progresses. This knowledge could then pave the way for new strategies in preventing and treating the condition. They also pointed out that the most effective disease model closely mimics the disease's underlying biological mechanisms. Given the many factors contributing to obesity, researchers have various approaches to creating these experimental models in animals, including manipulating neuroendocrine, dietary, and genetic factors. Ultimately, the choice of model should align with the specific environmental or genetic aspects being investigated.

Marques et al. (2015) [04] conducted a study comparing Wistar and Sprague-Dawley (SD) rats as models for obesity induced by a high-fat (HF) diet. Their findings indicated that the HF diet led to increased energy intake, weight gain, body fat percentage, the size of mesenteric fat cells, and circulating levels of adiponectin and leptin in both rat strains. Additionally, both groups exhibited reduced oral glucose tolerance. However, most of these effects were more pronounced or observed sooner in the Wistar rats. The researchers noted that Wistar rats consuming the HF diet had a greater food intake (data not presented), resulting in higher energy consumption throughout the study compared to SD rats on the same diet. As a consequence, Wistar rats experienced more significant weight gain, which was primarily attributed to the expansion of their adipose tissue.

Bautista et al. (2019) [05] detailed the biological processes and metabolic changes linked to obesity, specifically highlighting the monosodium glutamate (MSG)-induced obesity model. Their research indicated that in male mice with MSG-induced obesity, inflammation was more pronounced and levels of adiponectin were lower. Furthermore, they observed that glucose tolerance, insulin sensitivity, and redox balance were affected by age in both male and female mice. These results suggest that the metabolic changes in MSG-induced obesity are influenced by both gender and age. Consequently, they concluded that the MSG obesity model is useful for understanding the interplay between gender, aging, and metabolic alterations in obesity. Additionally, their review covered medicinal plants and their active compounds used in treating MSG-induced obesity, emphasizing the need for further research into the benefits and mechanisms of medicinal plants with known anti-obesity effects, given the model's significance.

Reza et al. (2020) [06] found that *Azadirachta indica* (*A. indica*) showed promising antidiabetic effects in both glucose tolerance tests and in a mouse model of alloxan-induced diabetes. They also observed that *A. indica* protected the liver against paracetamol-induced damage in mice, highlighting its biological and pharmacological significance. Their investigation into compounds from *A. indica* suggested that its antidiabetic action might involve increased insulin release and the regeneration of beta-cells. The antioxidant properties of these compounds likely also contribute to both the antidiabetic and liver-protective effects. The researchers recommended further molecular docking studies on other diabetes-related proteins and histopathological analysis of liver tissue to understand the impact of *A. indica* extracts on liver structure. They also suggested using different solvents to further separate the *A. indica* methanol extract (AiME) to identify more bioactive compounds. Ongoing research aims to uncover additional biomolecules and their mechanisms of action to better elucidate the antidiabetic and hepatoprotective properties of *A. indica*.

Hlaing et al. (2020) [07] studied the phytochemical constituents, total phenol contents and antioxidant potency of Flowers (F) and Pseudostems (P) of Aeginetia indica L.(Kauk- hlaing -ti). The phytochemical investigation indicated that alkaloids, carbohydrates, flavonoids , glycosides, organic acids, phenolic compounds , reducing sugars, saponins, steroids and terpenoids are present while starch and tannins are absent in both the samples. In addition, α-aminoacids are present in the flowers while they are absent in the pseudostems. AOAC method was used to determine the nutritional value of flowers and pseudostems of A.indica. The total phenol contents of different crude extracts was determined by UV-visible spectrophotometric using Folin-Ciocalteu reagent (FCR) method and garlic acid was used to construct standard calibration curve. Antioxidant activity of flowers and pseudostems of A.indica were also investigated by using DPPH assay method. The IC50 values of crude extracts were observed to be (31.66 µgmL-1) for F-EtOH, (82.63 µgmL-1) for F-H2O, (72.74 µgmL-1) for P-EtOH and (114.59 µgmL-1) for P-H2O respectively. Among the crude extracts, the order of radical scavenging activity was recorded as F-EtOH > P-EtOH > F-H2O >P-H2O. All extracts showed little activity upon comparison with the standard antioxidant vitamin C.

Kayode et al. (2023) [08] concluded that a common dietary enhancer, MSG, has a high tendency to induce development & progression of metabolic disorders such as obesity, cancer, hypertension and diabetes mellitus via various metabolic mechanisms involving induction of oxidative stress, hyperinsulinemia, dyslipidemia, hyperleptinemia, hyperphagia, GLUT transporters dysfunction and pro-proliferative action. Depending on the dosage, MSG can have both advantageous and detrimental effects, lower doses will enhance energy balance and homeostasis while excessive consumption may result in the initiation of metabolic disorders. Despite the concerns surrounding its safety, MSG is nevertheless still highly consumed globally. We suggest that using MSG as flavoring agent should be minimized while further research on the biochemical effects of chronic consumption by humans is highly recommended.

On-Nom et al. (2024) [09] aimed to extract optimized total phenolic content (TPC) in varying extraction conditions by using the RSM and the Box-Behnken design (BBD). Results indicated that an extraction temperature of 90 °C, 80% (*v*/*v*) aqueous ethanol, and 0.5% (*w*/*v*) solid-to-liquid ratio yielded the highest TPC at 129.39 mg gallic acid equivalent (GAE)/g dry weight (DW). Liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) identified the predominant phenolics as apigenin (109.06 mg/100 g extract) and luteolin (35.32 mg/100 g extract) with trace amounts of naringenin and rutin. Under the optimal extraction condition, plant extract exhibited antioxidant activities valuing 5620.58 and 641.52 µmol Trolox equivalent (TE)/g DW determined by oxygen radical absorbance capacity (ORAC) and FRAP assay, while the scavenging capacity in total radicals at 50% (SC_{50}) was determined to be 135.50 µg/mL using 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay. The plant extract showed the inhibitory activities in opposition to the key enzymes relevant to type II diabetes, obesity, and Alzheimer's disease, suggesting the potential for medicinal applications.

V. MATERIAL AND METHODS

All the chemicals excluding Orlistat are available at Shiva Institute of Pharmacy, Village Luhnoo Kanatain, P.O. Chandpur, Tehsil Sadar, Distt. Bilaspur, Himachal Pradesh, India.

VI. COLLECTION & AUTHENTICATION OF THE PLANT MATERIAL

Plants are available in the nearby Village Luhnu Kanaitan, P.O. Chandpur, Distt. Bilaspur, H.P. the area of lesser Himalayas and Shivalik ranges. Its authentication was performed by Mr. P.SURESH BABU, Asst. Profesor Botany, Department of the Botany, Government Degree College Kukatpally, Medchal District, Telangana State, on 11th November 2024 as per ICBN rules.

VII. EXPERIMENTAL ANIMALS

Animals of Species Sprague Dawley rats of age 8-9 weeks, gender female weighing 150-200g are used. Route of administration used is oral and number of animals used is 30. Each being is kept in a clean polypropylene cage with a standard temperature of 22±2 degrees Celsius, a 12-hour light/dark cycle and a relative humidity of 45%. They are also given unrestricted access to a standard pellet meal (Ashirwad Industries of Hindustan Lever, Punjab, India) and unlimited water. A week before to the experiment's commencement, the animals are acclimated to a clean, ventilated environment. Drinking water and food supplies are provided in easy accessibility throughout the procedure, only except for short time fasting where water is freely accessible but food supply is not, 12 hrs before starting the treatment.

The acute oral toxicity of ethanolic extract of Aeginetia *indica* is evaluated in rat according to the guidelines given by OECD 423. A single high dose of 5000 mg per kg per oral of plant crude extract is done by the oral route to six rats the crude drug is suspended in vehicle (distilled water). Before the fasting period started, the weight of rats is recorded and dose of crude extract is calculated as per body weight. After an hour of treatment food is provided to the animals. After 6 hours of treatment period, the rats are checked for any toxicity, daily for 14 days. Surviving animals are checked for mortality, change in physical appearance, pain, injury behavioral pattern and signs of illness daily during the time.

Table 2 Components of High-Fat Diet.

Ingredients	Quantity (gm/kg)
Normal rat pellet	375
Clarified butter	180
Hydrogenated vegetable fat	100
Bengal gram	265
Palm oil	20
Vitamin and mineral mix	60
D-Methionine	03
Yeast powder	01
Sodium chloride	01

VIII. STUDY DESIGN

The animals which are utilised in this experimentation are Sprague Dawley rats who are easy to handle. The animals are kept for acclimation in quarantine area of animal house to keep them comfortable in the same environment for Seven days before commencement of experiment. Adjustment of room temperature 22°±2 C and humidity 50-60% light cycle 12 hrs dark and 12 hr light is maintained. The animal experiments are approved by CPCSEA (Approval No. SIP/IAEC/2024-01/03). The animals are

handled in accordance with the standards of CPCSEA and IAEC. Total 30 Rats are weighed first and then randomly allocated in to 5 groups 6 rats in each group. The water and standard chew diet which include 13.67% fat, 65.3% carbohydrate, and protein 20.1% is given to normal group and other 4 groups are fed with high fat diet which include butter oil (190gm/kg), lard (310 gm/kg), casein (289.0gm/kg), sucrose (90.5gm/kg), cornstarch (207.3gm/kg), l-cystein (3.33gm/kg), cornoil (16.0gm/kg), cellulose (50gm/kg), dextrose (115.0gm/kg), vitamin mix (13.33g/kg), mineral mix (46.66 gm/kg).
The high fat diet includes carbohydrate 35%, protein 20 %, and 45% of fat, ad libitum. Body weight and food consumed by animals are recorded every week.

Group 1 (Negative Control): Rats which are provided with oral normal diet and 0.9% normal saline throughout the study.
Group 2 High fat rich diet control: include rats given with the high fat rich diet for 8 weeks
Group 3 (High fat diet + Test Drug in low dose) : is treatment group which is given with low dose of out plant extract which we have obtained from plant *Aeginetia Indica* once a day for 42 days
Group 4 (High fat diet + Test Drug high dose) : is treatment group which is given with high dose of out plant extract which we have obtained from plant *Aeginetia Indica* once a day for 42 days
Group 5 (High fat diet + Standard Orlistat 30 mg/kg) it includes the standard group which is given the standard drug Orlistat 30mg per kg body weight once a day. All groups are fed with the high fat rich diet except normal control and their weight is measured weekly.

When the experiment is over, the animals are weighed and killed. Sterile falcon tubes are used to hold the trunk blood, which is extracted using 23 G1 syringes. Centrifuging blood at 500xg for 15 minutes at 4ºC allowed for the collection of serum. Following that, it is kept at -80ºC until biochemical analysis. Half of the organ is prepared for histopathology and the other half is treated with liquid nitrogen and kept at -80ºC for further biochemical investigation.
Serum is collected by centrifugation of blood at 500×g for 15 min at 4ºC. These samples are stored at −80ºC until further biochemical analysis. The half of the organ is cryopreserved with liquid nitrogen & stored at -80ºC for subsequent biochemical analysis and the remaining part is processed for histology.

IX. POSSIBLE OUTCOMES

The general expected outcomes are:

1. Reduction in Body Weight.
2. Decrease in Adipose Tissue and Fat Accumulation.
3. Improvement in Lipid Profile : HDL, VLDL, LDL.
4. Improved Harmonal levels : Leptin & Adiponectin.
5. Reduction in Leptin Resistance
6. Reduction in Inflammatory Markers : IL6, TNFX
7. Behavioral changes such as decreased food intake (hypophagia) and increased energy expenditure.
8. Reduction in Liver and Kidney Damage

X. CONCLUSION

The study on the Antiobesity potential of "*Aeginetia Indica"* in HFD-induced obese rats is expected to conclude that this novel formulation is a safe, effective and patient-friendly alternative to control the obesity & its associated risks.
It is clear from the literature survey that a lot of work has been done in the field of Obesity & especially if we consider Aeginetia Indica, it has shown a promising outcome in regulating feed intake, modulating weight gain, improving lipid profiles. Though the researchers have used Aeginetia Indica several times but still there seems to be a lot of scope for new research work.

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