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SCREENING OF ANTI-OBESITY POTENTIAL OF *HIPPEASTRUM VITTATUM* FLOWER EXTRACT IN EXPERIMENTAL RATS

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ABSTRACT:

Obesity as an "abnormal or excessive fat accumulation that may impair health," further clarifying that "the fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. The phytochemicals and anti-obesity effect of *Hippaestrum vittatum* experimental rats was evaluated. A growing number of people are turning to the plant genus Hippeastrum, which contains several alkaloids, as a remedy for neurological disorders and neurodegenerative diseases. The most widely grown amaryllis in the world, *Hippeastrum vittatum*, is native to Central and South America, Brazil, and Peru. A botanist was able to correctly identify and certify *H. vittatum* flowers that were collected in the Unnao region. The obtained extract was determined for preliminary phytoconstituents and then evaluated for anti-obesity activity. Albino rats (both sexes) between 130 and 160 g were provided by the Animal House at the Institute of Pharmaceutical Sciences and Research in Unnao. Rats were divided in 5 groups i.e., control, disease control, standard test 1 and test 2. All the rodents were tested for parameters i.e., Body Weight (Gross), Weight of body organs i.e., Liver, Kidney, Brain, Lipid profile and total cholesterol level, Food consumption pattern, Blood glucose level and Antioxidant activity. In all the parameters, *H. vittatum* showed a significant modulation in order to confirm its anti-obesity potential. It lowered body weights of rodents when compared with the control at both the doses of 200mg/kg and 400mg/kg. Body organ weights also got decreased when measured at the spleen, liver and kidneys. Thus, by all the factors and protocols, it can be concluded that *H. vittatum* is an important and effective anti-obesity substance that be given in human beings after clinical trials demonstrating optimum safety and efficacy parameters. It would be very impactful with easier way of curing obesity, hypercholesterolemia and high-fat due to its wide availability and action.

Keywords: *Hippaestrum vittatum*, phytoconstituents, lipid profile, anti-obesity, antioxidant.

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INTRODUCTION

Obesity as an "abnormal or excessive fat accumulation that may impair health," further clarifying that "the fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended" (Salvador et al. 2017). This number is then used to place an individual into one of three categories: "underweight," "overweight," or "obese." The body mass index was developed by a Belgian mathematician and sociologist in the 1830s, but it is still commonly used today (Sadaf et al., 2021). Scientists have calculated that there were 641 million obese adults worldwide in 2014, up from an estimated 105 million in 1975 (Chunlan et al., 2018).

Obesity happening rates have risen globally in the last three decades, from 1980 to 2008, achieving a global prevalence of 10–14 percent among adults in 2008 (Finucane et al. 2011). Obesity prevalence is greater in upper-middle & high-income countries, but they are expected to rise quickly in poorer countries (Kelly et al. 2008). According to recent data from IDF, Kuwait is now among the top 7% of nations with the highest occurrence rate of adult obesity and it is in the top 3% of nations with the highest occurrence rate of diabetes (IDF, 2012). Genotype is thought to be responsible for roughly 40% of the variation in daily energy expenditure. There is therefore ample proof that heredity influences how one manages their body weight (Bouchard & Tremblay, 1990).

Plant profile

A growing number of people are turning to the plant genus Hippeastrum, which contains several alkaloids, as a remedy for neurological disorders and neurodegenerative diseases. It is composed of more than 70 species that can be found in tropical and subtropical areas of South America. Hippeastrum plants came in a variety of hybrids, and most present commercial hybrids are descended from *Hippeastrum vittatum*.

Taxonomy

Kingdom	: Plantae
Superorder	: Lilianae
Subclass	: Rosidae
Order	: Asparagales
Family	: Amaryllidaceae
Genus	: *Hippeastrum*
Species	: *vittatum*

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a. Leaves

b. Leaves

Fig 1. *Hippeastrum vittatum* plant

The most widely grown amaryllis in the world, *Hippeastrum vittatum*, is native to Central and South America, Brazil, and Peru. It was first transported to Europe in 1633 from South Africa at the Cape of Good Hope. Between the middle of the 19th century and the start of the 20th century, more than 50 Hippeastrum species were introduced to Europe. The Yangtze River in the south is the primary planting area for many of the current interspecific hybrid species, and many other potted ornamental. There are roughly 75 different species of amaryllis, which were first introduced in the early twentieth century. Amaryllis bulbs, roots, and flowers have been the subject of much investigation in recent years, with both domestic and foreign researchers isolating extracts from a variety of chemical components (Kim et al. 2006).

Chemical constituents

The novel alkaloids, tannins, glycosides etc. were discovered through analysis of an ethanolic fresh flower extract from *Hippeastrum vittatum* (Mishra et al. 2022)-

- Vittacarboline
- O-methylismine
- Ismine
- Lycorine
- Crinine
- Haemanthamine
- Narciclasine
- Galanthamine
- Tazettine

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MATERIALS AND METHODS

Experimental requirements

Hippaestrum vittatum, Wistar rats of both sexes, progesterone, distilled water, rotatory evaporator, weighing machine (digital) and ethanol.

Collection, authentication, and extraction of plant

A botanist was able to correctly identify and certify H. vittatum flowers that were collected in the Unnao region. The flowers are cleaned to remove any dust and dried in the shade or at room temperature. The flowers are dried and then ground into a coarse powder. Weighing the powder, we immerse it in ethanol (95%) for fifteen days while slowly stirring. The extract, which is dark and semisolid, is dried in a water bath. Actual yield is used to determine the extract's percentage (Maji, 2020).

Phytochemical study

To get better knowledge about the phytochemicals in the obtained extracts qualitative test alkaloids, terpenoids, proteins etc. were performed following standard chemical tests (Patel et al. 2012).

Saponins test

A modest amount of extract and water should be combined in a test tube for the foam test. When saponin is present, vigorous shaking produces foam that lasts for around 10 minutes.

Alkaloids

Chloroform is used to dissolve plant extract in the Dragendroff test. After the chloroform has been acidified and evaporated, add a few drops of Dragendroff's agent.

Mayer's test

Adding Mayer's reagent to 2–3 ml of filtrate yields ppt.

Wagner's test

When Wagner's reagent is added to 2–3 ml of filtrate, a reddish-brown colour develops. The presence of alkaloids in plant extracts was determined by adding a few drops of Hager's reagent and observing the resulting yellow precipitate.

Carbohydrate analysis

The Fehling's test:

Boiling for one minute a millilitre of each of Fehling's A and B solution mixtures. A similar quantity should be added to the solution of test extract. Immerse for about 5-10 minutes maximum. Carbohydrates are present as indicated by the appearance of a precipitate of orange red colour.

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Benedict's test

Combine test extract with Benedict's reagent in a test tube at a volume ratio of 1:1. Bring to a boil and let sit for five minutes. The consensus appears to be positive. The test solution's hue will be either yellow or red, depending on the concentration of disaccharide present.

Flavonoid Analysis

Ferric chloride test:

Mix a few drops of diluted ferric chloride with the extract's ethanol solution. The colour green represents the presence of flavonoids. Add 5ml (95% ethanol), a few drops of hydrochloric acid, and 0.5g (magnesium powder) to a dry extract to perform the Shinoda test. There's a hint of pink there. The yellow hue of test solutions is heightened after being treated with sodium hydroxide solution, but it disappears when a weak acid is added. Adding a few drops of lead acetate to the test solution causes a greenish-lemon precipitate to form.

Protein analysis

Biuret test:

Add 2 ml of the biuret solution, mix it well, and heat it in a water bath. Red or violet hues indicate the presence of proteins. Mix 3 ml of extract with a few drops of a 15 $CuSO_4$ solution and 4 percent NaOH. It gives off a pink/violated vibe.

Million's test: A reddish-brown precipitate is formed when a standard solution is added to Million's Reagent and then heated. The xanthoprotein test is boiling the test solution in concentrated nitric acid to create a yellow precipitate.

Ninhydrin test

It involves a reagent called ninhydrin, which gives the test solution a bluish tint.

Glycosides identification

Keller-Kelliani test:

The test solution was made by mixing two millilitres of ferric chloride solution with a few drops of glacial acetic acid. Sulfuric acid dripping down the sides of the test tube causes the top layer to turn blue-green, and then the two layers to separate and show a reddish-brown colour.

Amino acids test

Bring 3 ml of extract and 3 drops of a 5% Ninhydrin solution to a boil in a water bath, then let it sit for 10 minutes. The sky turns violet or blue. Warm 3 ml of extract and add 3 drops of Million's reagent to conduct the tyrosine test. The answer turns a deep crimson hue when mixed.

Steroid analysis

Add 3 milliliters of extract to 3 milliliters of acetic anhydride to perform the Liebermann reaction. Calm and comfortable Sulfuric acid in the form of a few drops Seems to be a blue colour.

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Preparation of animals

Albino rats (both sexes) between 130 and 160 g were provided by the Animal House at the Institute of Pharmaceutical Sciences and Research in Unnao. The animals are kept in a healthy environment with a room temperature of 25 degrees Celsius and a light/dark cycle of 12 hours. The rats are housed in a clean, humid environment (between 44 and 56 percent relative humidity) and fed a standard rodent diet with unlimited water. The animals are allowed free access to water up to an hour before the administrations (Bhajoni et al., 2016), but they continue to fast throughout the study.

Group design

Rodents were divided into 5 groups; n=6. The dosing was given for 25 days for each group as following-

Table. Group design with treatment

Group	Treatment
Control	Normal saline (20ml/kg, *p. o.*)
Disease control	Progesterone (20mg/kg, *s. c.*)
Standard	Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*)
Test 1	Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*)
Test 2	Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*)

Parameters

1. **Body weight**

   Each group's animals are weighed both before and after the medicine is given to ensure uniform dosing. The body's weight is measured both before and after the medicine is given.

2. **Blood sugar level**

   Blood glucose is estimated seven times at 0, 5, 10 and 15 days after the start of the administration of drugs. Blood sample is collected from the puncturing tail vein, and blood glucose is estimated using a blood glucometer made by Dr Morepen. This procedure is easy and authentic (Jung et al. 2021).

3. **Food consumption pattern**

   Food consumption behavior of rats are studied on days 5, 10 and 15. The rats are deprived of food/ high fat diet, 1 hour before to the experiment. After 30 minutes of progesterone ingestion, 10 g of rat pellets are given to groups of rats in their cages and food ingestion was recorded at every 0.5, 1, and 1.5 h intervals (Nderitu et al. 2017).

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Progesterone has been reported for potent obesity enhancing hormone for this it was used in the study.

4. **Weigh of organs**

After being sacrificed, all of the rats are given an incision. To further verify the effects on various organs, we weighed individual organs such the kidney, liver, and brain.

5. **Lipid profile and total cholesterol level**

Lipid profile including Total Cholesterol, Triglycerides, HDL, LDL and VLDL was conducted appropriately according to specified protocols. All the tests were performed by using their specific medical kits available in the market. Triglycerides level in blood plasma was also measured by its medical kit procured from the certified manufacturer and supplier (Dholi et al. 2011).

6. **Antioxidant activity**

DPPH method was used for evaluation of anti-oxidant activity. All the rats were starved for 16 hours after 25 days, and blood samples from the recto orbital plexus were taken. Using centrifuge, the serum was then separated to examine the antioxidants levels including SOD, CAT, TBARS & GSH.

Statistical analysis

The statistical data was analyzed using a two-tailed T test after an ANOVA was performed. The statistical analysis was performed using Sigma Stat pro3.3, and results were presented in terms of standard error of the mean. At the ≤P0.05 threshold of significance, the findings were accepted.

RESULTS AND DISCUSSION

Percentage yield

When calculated with theoretic yield the % yield of the herbal extract was found to be 64.67%.

Phytoconstituents

It has demonstrated for diverse phytoconstituents as follows-

Table 2. Phytochemicals of *H. vittatum* extract

Tests	Observation
Alkaloids	++
Saponins	+
Tannins	+
Proteins	+
Glycosides	++
Amino acids	++
Steroids	+

++= High +=Moderate

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ANTI-OBESITY POTENTIAL

Body weight determination

In order to confirm the anti-obesity potential of *Hippaestrum vittatum* that rats were taken and divided in 5 groups. The Group 1 was administered with Normal saline (20ml/kg, *p. o.*), group 2 with Progesterone (20mg/kg, *s. c.*), group 3 treated with Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*) and group 4 fed with Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*) whereas group 5 administered Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*) for once per day up to 25 days.

Table 4.1 Body weight of *Hippaestrum vittatum* treated rats

Treatment	**Body weight (g)**	
	Before	***after***
Normal saline (20ml/kg, *p. o.*)	63.19±0.20**	65.29±0.12**
Progesterone (20mg/kg, *s. c.*)	64.32±0.51***	67.25±0.289**
Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*)	69.19±0.42**	66.29±0.39***
Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*)	67.29±0.19**	69.28±0.27**
Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*)	64.27±0.28**	68.12±0.18**

N=6 significant results at P≤0.05;
data shown as mean standard error of the mean

In body weight determination, body weight of different rat was determined before treatment and after treatment for 25 days. In this context, normal saline fed group showed a significant increase in body weight after treatment which was 63.19±0.20**g as before and 65.29±0.12**g as after. Progesterone showed a remarkable increase in body weight as 67.25±0.289**g which was 64.32±0.51***g before the treatment.

Orlistat exhibited marked decrease in body weight even with progesterone exposure. *Hippaestrum vittatum* demonstrated body weight as 69.19±0.42**g and 66.29±0.39***g after the treatment, at dose of 200mg/kg and 400mg/kg, respectively that was significant decrease in body weight. Thus, this maintained body weights of mice while given with high fat diet.

Weight of organs

In the way to confirm the anti-obesity potential of *Hippaestrum vittatum* that rats were taken and divided in 5 groups. The Group 1 was administered with Normal saline (20ml/kg, *p. o.*), group 2 with Progesterone (20mg/kg, *s. c.*), group 3 treated with Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*) and group 4 fed with Progesterone (20mg/kg, *s. c.*) + EFHV

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(200mg/kg, *p. o.*) whereas group 5 administered Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*) for once per day up to 25 days.

Rats were anesthetized with diethyl ether. Then they were sacrificed with cervical dislocation method, prior dissecting different organs to weight.

Following picture demonstrates the dissection of rats-

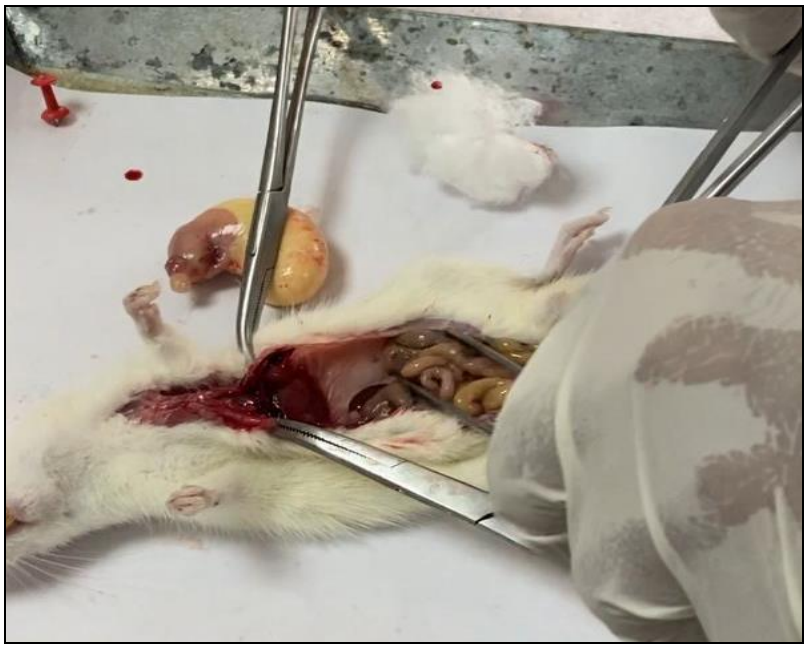

Fig. Dissecting rat

Table 2. Weight of organs

Treatment	Spleen (g)	Kidney (g)	Liver (g)
Normal saline (20ml/kg, *p. o.*)	0.24±0.17*	1.49±0.26*	7.594±0.20*
Progesterone (20mg/kg, *s. c.*)	0.43±0.11**	1.82±0.71**	9.19±0.18**
Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*)	0.34±0.28*	1.57±0.38**	8.16±0.31**
Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*)	0.39±0.26*	1.75±0.13**	8.73±0.28**
Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*)	0.36±0.37***	1.37±0.20***	8.41±0.22***

N=6 significant results at P≤0.05;

data shown as mean standard error of the mean

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In determination of weight of organs, control group showed normal weights of spleen, kidney and liver when estimated. The progesterone fed mice exhibited an increase in body weights as 0.47±0.20**g, 1.82±0.71**g and 9.19±0.18**g of spleen, kidney and liver, respectively. Orlistat fed group significantly decreased weights of concerning organs. Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*) showed weights i.e., 0.39±0.26*g (spleen), 1.75±0.13**g (kidney) and 8.73±0.28**g (liver) whereas, Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*) significantly decreased weight of organs of spleen, kidney and liver as 0.36±0.37***g, 1.37±0.20***g and 8.41±0.22***g respectively.

Total cholesterol level

In evaluation of the anti-obesity potential of *Hippaestrum vittatum* that rats were taken and divided in 5 groups. The Group 1 was administered with Normal saline (20ml/kg, *p. o.*), group 2 with Progesterone (20mg/kg, *s. c.*), group 3 treated with Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*) and group 4 fed with Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*) whereas group 5 administered Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*) for once per day up to 25 days.

Table 4.3 Estimation of Total Cholesterol (TC)

Treatment	TC (mg/dl)				
	Day 1	Day 3	Day 5	Day 10	Day 15
Normal saline (20ml/kg, *p. o.*)	109.19±3.11*	104.31.20**	106.29±1.28**	111.10±3.65*	119.17±2.26**
Progesterone (20mg/kg, *s. c.*)	157.13±2.14**	154.10±3.17**	153.14±2.40**	154.18±3.17**	159.30±2.54**
Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*)	114.20±3.19**	116.18±2.16**	118.13±5.20*	129.29±3.29**	123.20±2.52**
Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*)	148.10±3.18**	151.29±3.93***	154.62±2.45**	156.38±2.27**	143.19±2.14**
Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*)	127.13±2.19**	130.20±4.17**	134.13±2.71**	137.28±2.39**	132.12±2.46**

N=6 significant results at P≤0.05;
data shown as mean standard error of the mean

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Total cholesterol level was estimated in day 1, 3, 5, 10 and 15 in all the treatment group of animals. Total cholesterol was estimated in Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*) administered rat as 127.13±2.19**mg/dl, 130.20±4.17**mg/dl, 134.13±2.71**mg/dl, 137.28±2.39**mg/dl and 132.12±2.46**at the day 1, 3, 5, 10 and 15, respectively which was comparable to Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*) treated group as 114.20±3.19**mg/dl (day 1), 116.18±2.16**mg/dl (day 3), 118.13±5.20*mg/dl (day 5), 129.29±3.29**mg/dl (day 10) and 123.20±2.52**mg/dl (day 15).

The progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*) administered rat exhibited total cholesterol level as 148.10±3.18**mg/dl, 151.29±3.93***mg/dl, 154.62±2.45**mg/dl, 156.38±2.27**mg/dl and 143.19±2.14**at the day 1, 3, 5, 10 and 15 respectively that was different when compared with the control and progesterone fed rodents. Therefore, at both the doses *Hippaestrum vittatum* showed anti-obesity potential in mice.

Triglyceride level

In evaluation of the anti-obesity potential of *Hippaestrum vittatum* that rats were taken and divided in 5 groups. The Group 1 was administered with Normal saline (20ml/kg, *p. o.*), group 2 with Progesterone (20mg/kg, *s. c.*), group 3 treated with Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*) and group 4 fed with Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*) whereas group 5 administered Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*) for once per day up to 25 days.

Triglyceride level was estimated in the day 1, 3, 5, 10 and 15 in all the treatment group of animals. Triglyceride level was estimated in progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*) administered rat as 110.21.23**mg/dl, 117.29±2.39**mg/dl, 124.18±2.51**mg/dl, 129.27±2.18**mg/dl and 123.19±2.12**mg/dl at the the day 1, 3, 5, 10 and 15, respectively which was comparable to Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*) treated group as 113.27±3.19**mg/dl (day 1), 116.28±2.62**mg/dl (day 3), 119.25±2.18**mg/dl (day 5), 126.26±2.38***mg/dl (day 10) and 119.27±2.16**mg/dl (day 15).

Whereas, Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*) administered rat exhibited Triglyceride level as 112.46±2.42**mg/dl, 117.16±3.28*mg/dl, 122.24±3.20**mg/dl, 126.17±2.230**mg/dl and 1.27±2.16**mg/dl at the day 1, 3, 5, 10 and 15 respectively that was different when compared with the control and progesterone fed rodents. Therefore, at both the doses *Hippaestrum vittatum* showed anti-obesity potential in terms of lowering the triglyceride level in mice.

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Table 4.4 Estimation of Triglyceride level

Treatment	Triglyceride level (mg/dl)				
	Day 1	Day 3	Day 5	Day 10	Day 15
Normal saline (20ml/kg, *p. o.*)	109.19±2.19**	111.34±2.28**	114.15±2.52**	112±2.27***	117.13±2.14**
Progesterone (20mg/kg, *s. c.*)	112.17±2.30**	115.39±3.38**	119.20±2.35**	137.19±2.32***	152.19±2.19**
Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*)	113.27±3.19**	116.28±2.62**	119.25±2.18**	126.26±2.38***	119.27±2.16**
Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*)	112.46±2.42**	117.16±3.28*	122.24±3.20**	126.17±2.230**	1.27±2.16**
Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*)	110.21.23**	117.29±2.39**	124.18±2.51**	129.27±2.18**	123.19±2.12**

N=6 significant results at P≤0.05;
data shown as mean standard error of the mean

Food consumption pattern

In evaluation of the anti-obesity potential of *Hippaestrum vittatum* that rats were taken and divided in 5 groups. The Group 1 was administered with Normal saline (20ml/kg, *p. o.*), group 2 with Progesterone (20mg/kg, *s. c.*), group 3 treated with Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*) and group 4 fed with Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*) whereas group 5 administered Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*) for once per day up to 25 days.

Food consumption pattern was also estimated in the day 1, 3, 5, 10 and 15 in all the treatment group of animals. Food consumption pattern was estimated in Progesterone (20mg/kg, *s. c.*) +

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EFHV (400mg/kg, *p. o.*) administered rats as 104.12±0.11**mg/dl, 105.49±0.10***mg/dl, 103.19±0.54**mg/dl, 99.13±0.35** mg/dl and 96.24±0.38**mg/dl at the day 1, 3, 5, 10 and 15, respectively which was comparable to progesterone + Orlistat treated group as 97.13±0.10*mg/dl (day 1), 99.21±0.73*mg/dl (day 3), 93.12±0.65**mg/dl (day 5), 88.12±0.43***mg/dl (day 10) and 85.20±0.11*mg/dl (day 15).

Table 4.5 Food consumption pattern after treatment

Treatment	Blood sugar level (mg/dl)				
	Day 1	Day 3	Day 5	Day 10	Day 15
Normal saline (20ml/kg, *p. o.*)	86.18±0.34*	84.21±0.27**	87.13±0.11*	86.21±0.43**	83.27±0.46**
Progesterone (20mg/kg, *s. c.*)	98.26±0.11**	111.26±0.31*	122.18±0.34**	121.18±0.43**	139.24±0.45*
Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*)	97.13±0.10*	99.21±0.73*	93.12±0.65**	88.12±0.43***	85.20±0.11*
Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*)	103.25±0.15**	104.16±0.05**	105.21±0.45**	94.12±0.45**	86.28±0.34**
Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*)	104.12±0.11**	105.49±0.10***	103.19±0.54**	99.13±0.35**	96.24±0.38**

N=6 significant results at P≤0.05;
data shown as mean standard error of the mean

Whereas, Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*) administered mice exhibited food consumption pattern as 103.25±0.15**mg/dl, 104.16±0.05**mg/dl, 96.51±0.34**mg/dl, 94.12±0.45**mg/dl and 86.28±0.34**mg/dl at the day 1, 3, 5, 10 and 15

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respectively that was different when compared with the control and progesterone fed rodents. Therefore, at both the doses *H. vittatum* showed decreased food consumption pattern.

Estimation of blood glucose level

The Group 1 was administered with Normal saline (20ml/kg, *p. o.*), group 2 with Progesterone (20mg/kg, *s. c.*), group 3 treated with Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*) and group 4 fed with Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*) whereas group 5 administered Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*) for once per day up to 25 days.

Blood Glucose Level was estimated in 6 hours, 12 hours, 18 hours, 24 hours and 48 hours in all the treated rats. Blood Glucose Level was estimated in Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*) administered rat as 87.37±0.17**mg/dl, 91.34±0.19***mg/dl, 93.34±0.24***mg/dl, and 87.35±0.15**mg/dl at the 12, 18, 24 and 48 hours, respectively which was comparable to progesterone + Orlistat treated group as 85.27±0.85*mg/dl (12 hours), 87.70±0.34**mg/dl (18 hours), 89.46±0.82**mg/dl (24 hours) and 86.32±0.64**mg/dl (48 hours).

Whereas, Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*) administered rats exhibited Blood Glucose Level as 89.46±0.11**mg/dl, 91.32±0.18***mg/dl, 91.43±0.45**mg/dl and 93.43±0.53**mg/dl at the 12, 18, 24 and 48 hours, respectively that was different when compared with the control and progesterone fed rats.

Therefore, at both the doses *H. vittatum* showed anti-obesity potential in terms of lowering the blood sugar level.

Reduction in the levels of proteins, glucose- blood plasma indicate for the anti-obesity activity of the *H. vittatum*. It confirms that regulation of insulin becomes normal or increases the sensitivity of Tyrosine Kinase receptor subtypes for better binding and opening the glucose transporters. Thus, it facilitates the release of glucose molecules for better delivery at the targeted organs and produce the energy in terms of ATP for proper metabolism cycles of tissues. Its action might be based on the sensitization of receptors in Type 2 DM or insulin release in Type 1 DM.

Table 4.6 Estimation of blood sugar level

Treatment	Blood sugar level (mg/dl)				
	6 hours	12 hours	18 hours	24 hours	48 hours
Normal saline (20ml/kg, *p. o.*)	85.29±0.34*	86.54±0.11*	89.23±0.12**	86.24±0.10*	84.28±0.19*
Progesterone (20mg/kg, *s. c.*)	93.19±0.11**	97.18±0.10*	106.16±0.75***	111.35±0.16**	129.13±0.66*

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Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*)	84.25±0.24*	85.27±0.85*	87.70±0.34**	89.46±0.82**	86.32±0.64**
Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*)	87.23±0.23***	89.46±0.11**	91.32±0.18***	91.43±0.45**	93.43±0.53**
Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*)	84.26±0.24***	87.37±0.17**	91.34±0.19***	93.34±0.24***	87.35±0.15**

N=6 significant results at P≤0.05;
data shown as mean standard error of the mean

Antioxidant activity

Hippeastrum vittatum was also studied for antioxidant activity. Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*) administered rats showed SOD level as 0.21±0.01***U/µg, CAT level as 11.35±1.32***nM/µg, TBARS as 0.82±0.02***nM of MDA/mg and GSH as 4.06±1.32µM/µg of protein.

Moreover, the effect was observed as dose-dependent because higher shown better results in contract to lower dose. Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*) treated rats exhibited TBARS as 1.09±0.02***nM of MDA/mg.

Table 4. Estimation of antioxidant activity

Treatment	SOD (U/µg of protein)	CAT (nM of H_2O_2/min/µg of protein)	TBARS (nM of MDA/mg of protein)	GSH (µM/µg of protein)
Normal saline (20ml/kg, *p. o.*)	0.26±0.03	13.54±2.03	0.54±0.03	7.63±1.40
Progesterone (20mg/kg, *s. c.*)	0.42±0.05	19.34±1.54	0.47±0.04	9.34±2.43
Progesterone (20mg/kg, *s. c.*) + Orlistat (50mg/kg, *p. o.*)	0.11±0.04	8.46±0.30	1.51 ±0.03	4.06±1.32

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Progesterone (20mg/kg, *s. c.*) + EFHV (200mg/kg, *p. o.*)	0.21±0.01***	11.35±1.32***	0.82±0.02***	6.41±1.52***
Progesterone (20mg/kg, *s. c.*) + EFHV (400mg/kg, *p. o.*)	0.17±0.04***	11.32±0.38***	1.09±0.02***	4.34±1.37***

N=6 significant results at P≤0.05;
data shown as mean standard error of the mean

In all the parameters, *H. vittatum* showed a significant modulation in order to confirm its anti-obesity potential. It lowered body weights of rodents when compared with the control at both the doses of 200mg/kg and 400mg/kg. Body organ weights also got decreased when measured at the spleen, liver and kidneys.

Food consumption pattern was reduced in the *H. vittatum* fed groups when compared with the control and HFD groups that refers that it is helpful in maintaining total energy level and thus obesity- a disturbed state of body that calls for diverse types of problems associated with health.

It is also linked with managing the total cholesterol and triglycerides and weights of organs. When food consumption is abandoned then it's obvious to control the body weight as in gross as well the weight of different organs.

In results, it significantly exhibited anti-obesity potential by showing the decreased body weight, body organs, total cholesterol, triglyceride levels, food consumption pattern and blood sugar level.

CONCLUSION

As the research concerns with evaluation of antiobesity role of *H. vittatum* in different experimental protocols among rodents. Decrease in body weight is an important parameter of anti-obesity potential, in this study, *H. vittatum* was successfully found active in lowering the body weights (gross) of mice. When observed body organs, they also decreased weights of liver, spleen and kidneys. This action might be due to lowering the energy levels thus controlling the weights.

Thus, by all the factors and protocols, it can be concluded that *H. vittatum* is an important and effective anti-obesity substance that be given in human beings after clinical trials demonstrating optimum safety and efficacy parameters. It would be very impactful with

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easier way of curing obesity, hypercholesterolemia and high-fat due to its wide availability and action.

Future impact

This study suggests that *H. vittatum* might be prescribed as a well-known herbal formula for managing obesity and reducing the bad cholesterol and thus refining the better health for longer lives, after more successful researches.

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Nil.

CONFLICT OF INTEREST

None.

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