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Research Article

Preclinical Evaluation of Herbal Formulation Against Progesterone-Induced Obesity in Mice

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ABSTRACT

The present study evaluated the antiobesity potential of a formulation. As obesity is a chronic metabolic condition and leads to various co-morbidities, hence there is a need to find herbal promising treatment with maximum potential and minimum side effects. Marketed allopathic drugs have side effects; hence, here is a switch to herbal treatment. Obesity was triggered *via* subcutaneous administration of progesterone over a 28-day period, during which both a polyherbal formulation and a standard treatment were given. Essential physical measurements were documented, along with serum parameters such as total cholesterol, triglycerides, LDL, HDL, and VLDL. Moreover, liver function enzymes (SGPT and SGOT) and glucose levels were evaluated, and a histopathological analysis of adipose tissue was conducted. The results demonstrated that the polyherbal formulation significantly reduced obesity-related parameters, such as VLDL, total cholesterol, HDL, triglycerides, and LDL, in the treatment group. These findings suggest that the polyherbal formulation effectively counteracts progesterone-induced obesity.

Introduction

Multiple causative variables characterize obesity. Self-rated health is the second-most preventable cause of death after smoking. This activity assesses and consolidates knowledge regarding the disorder's causes, pathophysiology, presentation, and complications and affirms the collaborative approach to obesity management.[1]

In 2016, the World Health Organization (WHO) reported that over 1.9 billion individuals in the age group of 18 and above were overweight, with over 650 million of those persons being obese. Over the past 40 years, there has been a significant rise in overweight and obesity among kids and teenagers (5–19 years old).[2]

WHO reports that there are 300 million individuals who are clinically obese and over one billion overweight adults worldwide, indicating that obesity has reached epidemic proportions. Obesity and overweight are not just adult problems; research from previous decades points to a rise in childhood and teenage obesity as well as reports from younger age groups. Adolescent overweight prevalence was found in many Indian research to range from 2.2 to 25.0% (Figs 1 & 2).[3]

The morbidity and mortality associated with obesity make its control and treatment necessitate several resources such as pharmacological medications, proper diet and exercise, and costs of training, among others. Thus, there are five different types of mechanisms or strategies for weight loss.[4]

Decreasing food intake, inhibiting nutrient absorption, elevating thermogenesis, terminating with protein or fat metabolism or storage, altering the one controlling the body weight in the central region of the brain (Fig. 3).[5]

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Interventions in Lifestyle [8]

Behavioral therapy

Cognitive-behavioral therapy (CBT) and other programs that emphasize behavior modification are effective in helping people establish healthier routines.

Dietary adjustments

Low-carb diets and the Mediterranean diet are two structured weight-loss diets that have proven promise. Whole foods, fruits, vegetables, lean meats, and healthy fats are prioritized.

Physical exercise

It is essential to increase physical exercise. According to recent recommendations, combining strength and aerobic training is beneficial.

In line with this Ayurvedic tradition, our selected plants, *Zingiber officinale, Allium sativum, Glycyrrhiza glabra, Trigonella foenum-graecum, Gymnema sylvestre, Myristica fragrans*, and *Withania somnifera*, have been traditionally claimed for their significant potential in treating obesity. With its diverse bioactive compounds, the formulation may help overcome obesity-related co-morbidities. This plant forms a cornerstone of our project. Additionally, the polyherbal treatment may act synergistically, offering a broad therapeutic spectrum. It will increase patient compliance, as polyherbal treatment has less toxicity and balanced efficacy. The herbal treatment has less resistance and is more adaptable, and these benefits are also cost-effective.

Thus, the present study, titled "Preclinical evaluation of herbal formulation for its antiobesity activity.", aims to explore and validate the pharmacological benefits of polyherbal formulation in obesity management.

Material And Methods

Animals

Female Swiss albino mice (20–25 g) were used for progesterone-induced obesity study. Animals were procured from the National Institute of Biosciences, Pune,

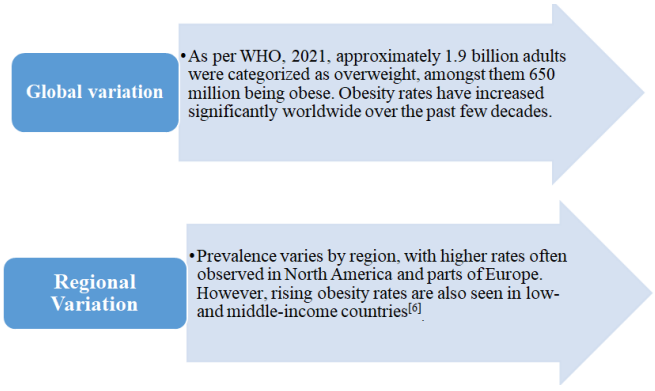


Fig. 1: Prevalence of Obesity

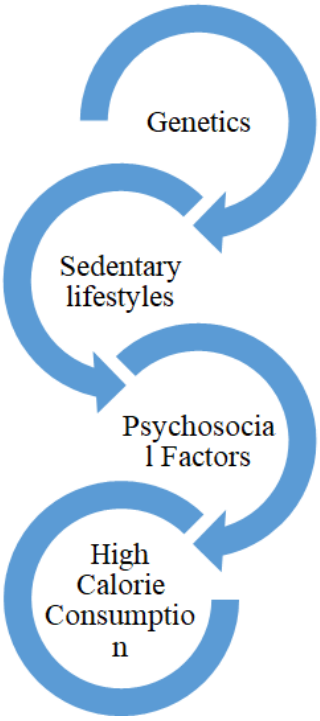


Fig. 2: Risk factors associated with obesity [7]

under IAEC approval 1554/PO/S/11/CCSEA. All animals were kept under normal climatic conditions, which include a 12-hour light-dark cycle, at 25 ± 2°C and 45 to 55% relative humidity. [12]

Plant Material

Collection and identification of medicinal plants

Z. officinale, A. sativum, G. glabra, T. foenum graecum, G. sylvestre, M. fragrans, W. somnifera were procured from a local market and were authenticated by Dr. Meenal D. Lad, HOD, Department of Dravyagunavigyan, Nigdi, Pune dated 15th April 2022.

Extraction of plant materials of selected plant by maceration process

The coarse material was kept into a container with solvent for a minimum of three days. Periodically, the content is stirred. Filtration or decantation was used to separate the micelle from marc. [13]

Preparation of formulation (Syrup)

Prepared syrup by using honey and distilled water. Firstly, all the extracts were taken in their specific quantities as per the standard quantity and mixed well with each other by adding a small amount of water and honey. The volume was adjusted to a sufficient quantity of water, 100 mL.[14,15]

Acute oral toxicity study

OECD guidelines 420 and 423[12, 16] were used to assess acute oral toxicity. The extract was found to be safe and effective at 2000 mg/kg.

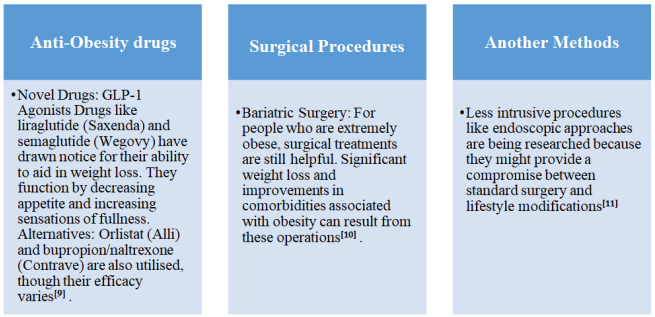


Fig. 3: Current treatment strategies to control obesity

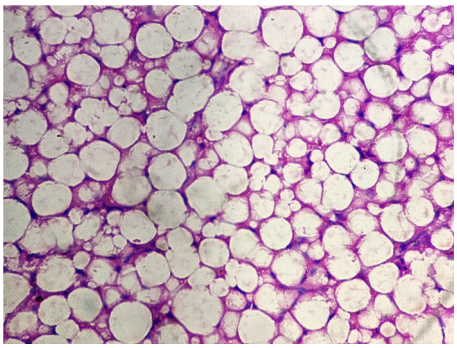
METHODS

Progesterone-induced obesity in female mice

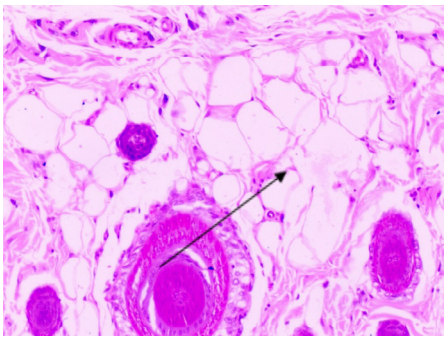
About 30 Swiss albino female mice in the weight range of 20 to 25 g were randomly divided into five groups, consisting of six animals each and subjected to respective treatments (Table 1).[17]

Progesterone was mixed into arachis oil and administered subcutaneously (10 mg/kg) after 30 minutes of administration of respective treatment of groups II to V.

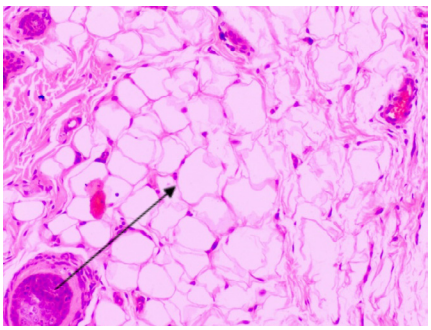
The physical parameters, i.e., body weight, abdominal circumference, and BMI (body mass index), were calculated.



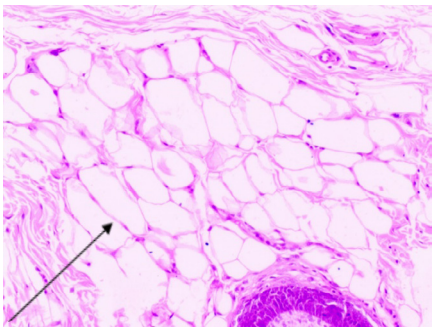
Control group- No abnormality detected adipose tissue, H&E 10x



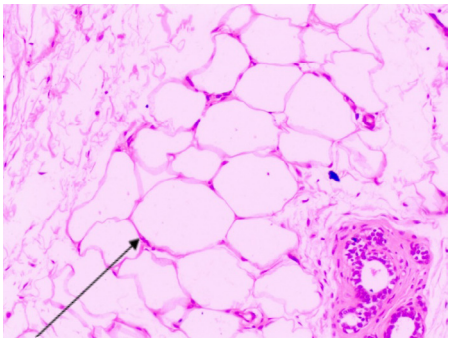
Induction group- Moderate increase size adipocytes and coalescing adipocytes (Arrow) in adipose tissue H&E 10x



Standard group- Minimal increase of adipocytes (arrow) adipose tissue, H&E 10X



Test group- 1 Mild increase of adipocytes (arrow) adipose tissue H&E 10X



Test group 2- Mild increase of adipocytes (Arrow) adipose tissue, H&E 10X

Fig. 4: Histopathology observation of adipose tissue

Table 1: Treatment schedule

Group	*Nomenclature of group*	*Treatment assigned*	*Duration*
I	Normal control	Free Access to water and food	1st to 28th day
II	Progesterone induction control	Distilled water 1-mL /100 gm, and progesterone 10 mg/kg, s.c.	1st to 28th day
III	Reference standard	Orlistat 20 mg/kg, p.o., and progesterone 10 mg/kg, s.c.	1st to 28th day
IV	PHF- low dose	PHF 200 mg/kg, p.o., and progesterone 10 mg/kg, s.c.	1st to 28th day
V	PHF-high dose	PHF 400 mg/kg, p.o., and progesterone 10 mg/kg, p.o	1st to 28th day

Table 2: Effect of PHF on progesterone induced alterations in physical parameters

Groups	*Body weight (gm)*					*Abdominal circumference (cm)*		*BMI (gm/cm²)*	
	0th day	*7th day*	*14th day*	*21st day*	*28th day*	*Start*	*End*	*Start*	*End*
Normal control	21.83 ± 0.6	22.7 ± 0.58	23.08 ± 0.38	24.08 ± 0.4	25 ± 0.77	5.1 ± 0.082	5.38 ± 0.133	69.62 ± 1.653	77.04 ± 2.141
Progesterone Induction control	22.17 ± 0.4 ^{ns}	25.52 ± 0.68 ^{ns}	26.5 ± 0.7260$^{\#\#}$	28.17 ± 0.9$^{\#\#}$	31.58 ± 0.77 $^{\#\#\#}$	5.13 ± 0.062^{ns}	6.3 ± 0.0966$^{\#}$	83.5 ± 1.76^{ns}	108.27 ± 2.16 $^{\#\#\#}$
Ref. Std Orlistat 20 mg/kg p.o.	22.5 ± 0.5 ^{ns}	25.02 ± 0.74^{ns}	25.58 ± 0.65^{ns}	25.9 ± 0.53 ^{ns}	24.85 ± 0.55 ***	5.11 ± 0.0477^{ns}	5.6 ± 0.0633^{ns}	85.97 ± 2.097^{ns}	98.51 ± 1.71 **
PHF 200 mg/kg p.o.	22.17 ± 0.95^{ns}	24.67 ± 0.62^{ns}	25.2 ± 0.64^{ns}	25.17 ± 0.54*	24.85 ± 0.51***	5.13 ± 0.0615^{ns}	5.5 ± 0.0365*	84.93 ± 4.234^{ns}	95.51 ± 1.552 ***
PHF 400 mg/kg p.o.	22.5 ± 0.85^{ns}	24.75 ± 0.93^{ns}	25.03 ± 0.81^{ns}	25.23 ± 0.67*	23.82 ± 0.57 ***	5.15 ± 0.056^{ns}	5.42 ± 0.0833^{ns}	84.11 ± 2.571^{ns}	92.12 ± 1.755 ***

After the 28th day of the study, blood samples were collected by the retro-orbital process for evaluation of serum parameters, including glucose, HDL, LDL, VLDL, triglycerides, total cholesterol, SGPT, SGOT, and the atherogenic index. For the histopathological study, isolated adipose tissues were preserved in 10% formaldehyde solution.

Statistical Analysis

Tukey's Kramer test was used for statistical analysis. *p < 0.05, **p < 0.01, ***p <0.001, ^{ns}p >0.05 when compared to progesterone induction control group.

Results

Physical Parameters

Effect of PHF on physical parameters is tabulated in Table 2.

Body weight

Group II exhibited significant (p <0.01, p <0.001) elevation in the body weight of mice on the 14th day, 21st day and 28th day.

PHF treatment showed a significant (p < 0.05, p < 0.001) and equipotent reduction in increased body weight on the 21st and 28th days compared to group II. Reference standard orlistat 20 mg/kg exhibited significant (p <0.001) body weight reduction on 28th day as compared to progesterone induction group II.

Abdominal circumference

On the 28th day of the study, abdominal circumference was significantly (p <0.05) increased in mice of group II. Group IV PHF increased abdominal circumference significantly (p <0.05).

BMI

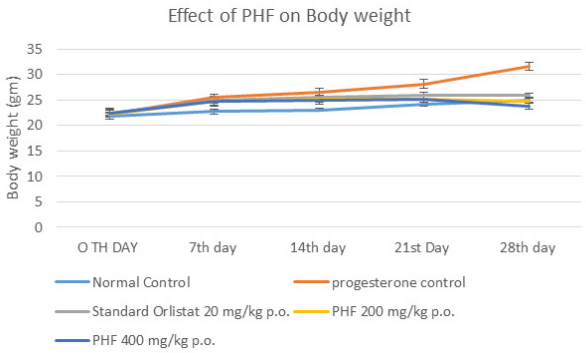
The progesterone-induced group (Group II) exhibited a significant increase (p < 0.001) in BMI on the 28th day of the study. The BMI was significantly increased (p <0.001) in groups IV & V PHF. The BMI of the reference standard orlistat-treated group at a dose of 20 mg/kg was significantly increased (p < 0.01) when compared with the progesterone induction control group.

Histopathology Observation

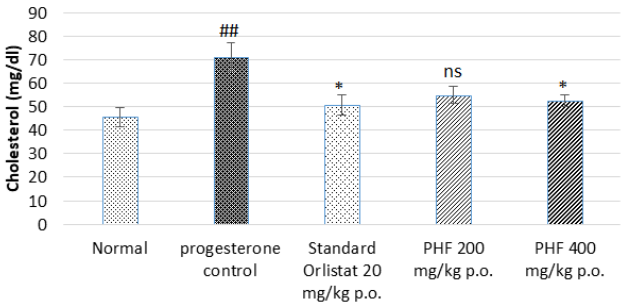
Histopathology observation of adipose tissue is mentioned in Fig. 4.

Effect of PHF on Progesterone-Induced Alterations in Lipid Profile

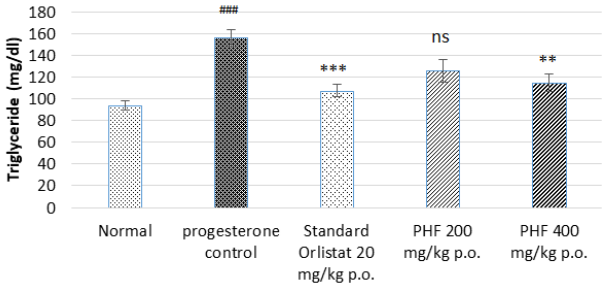
Effect of PHF on lipid profile is mentioned in Fig. 5A and B. The lipid profile parameters excluding HDL were significantly (p < 0.01, p < 0.001) increased in group



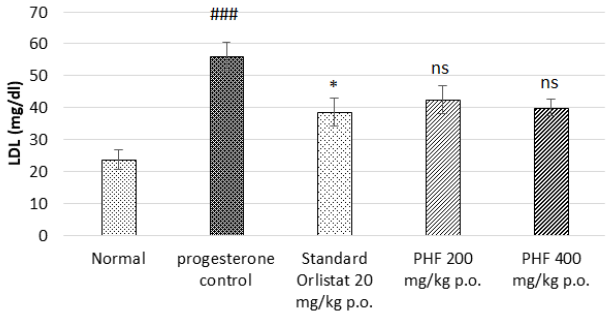
Graph 1: PHF effect on body weight



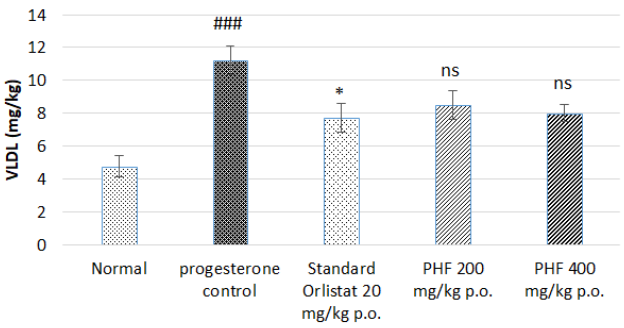
Graph 2: PHF effect on cholesterol



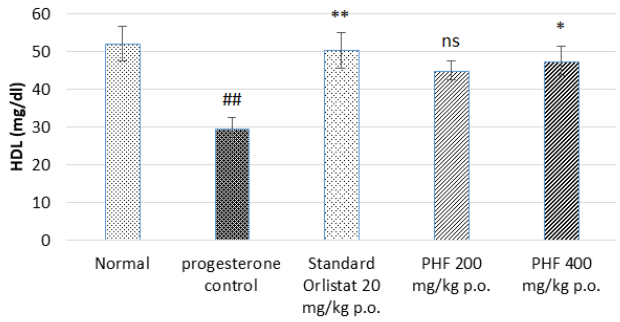
Graph 3: PHF effect on triglyceride level



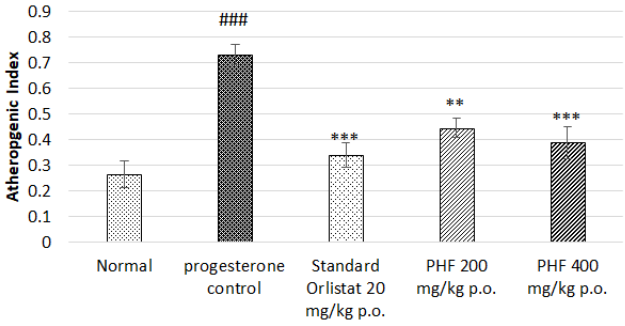
Graph 4: PHF effect on LDL



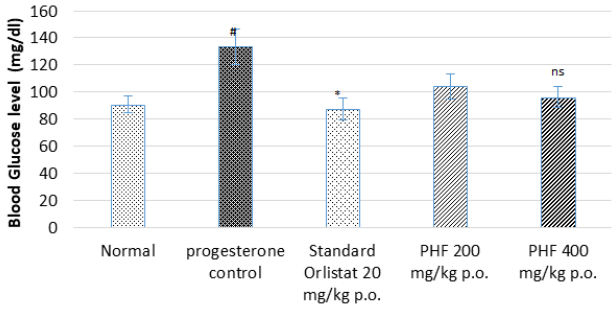
Graph 5: PHF effect on VLDL



Graph 6: PHF effect on HDL (mg/dl)

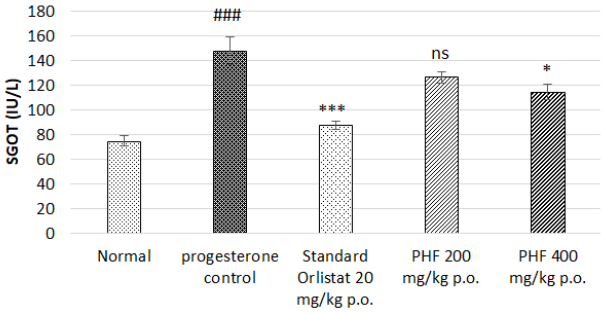


Graph 7: PHF effect on atheropgenic index

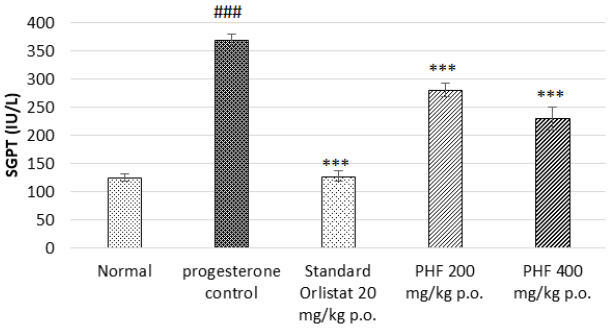


Graph 8: PHF Effect on Blood Glucose level

Fig 5A: Effect of PHF on body weight, blood glucose level and lipid profile



Graph 9: PHF Effect on SGOT



Graph 10: PHF Effect on SGPT

Fig 5B: Effect of PHF on liver profile

II. All these lipid profile parameters were significantly decreased ($p < 0.001$) in standard group III. PHF group V at dose of 400 mg/kg p.o. also showed significant reduction ($p < 0.05$, $p < 0.01$, $p < 0.001$) in cholesterol, triglyceride and atherogenic index, respectively. All lipid parameter alterations were not significantly restored in group IV, excluding the atherogenic index parameter.

The level of HDL-cholesterol was significantly ($p < 0.01$) decreased in group II, and its level was significantly ($p < 0.01$, $p < 0.05$) restored in the reference standard orlistat-treated group III and PHF-treated group V when compared to group II.

Effect of PHF on Progesterone-Induced Alterations in Liver Profile

The effect of PHF on the liver profile is mentioned in Fig. 5B. Liver serum enzyme SGPT & SGOT levels were increased significantly ($p < 0.001$) in group II, whereas significant ($p < 0.001$) equipotent reduction in SGPT was found in the PHF and standard group. The SGOT level was not significantly reduced by PHF at a dose of 200 mg/kg, whereas PHF at a dose of 400 mg/kg p.o. and standard orlistat at 20 mg/kg p.o. showed significant reduction ($p < 0.05$, $p < 0.001$) in SGOT level, respectively.

Effect of PHF on Progesterone-Induced Alterations in Blood Glucose Level

The glucose level was found to be significantly increased ($p < 0.05$) in group II, whereas it was significantly decreased ($p < 0.05$) in group III, treated with orlistat, in an equipotent manner. PHF-treated group-IV & V reduced elevated level of glucose as compared to normal control group-I but not significantly.

Discussion

Progesterone, secreted primarily by the corpus luteum, plays a crucial role in preparing the body for pregnancy and maintaining gestation. It exerts its effects through progesterone receptors, which are present in various tissues, including the hypothalamus and adipose tissue. As hormones have a huge impact on our body, progesterone this also responsible for obesity production. Basically, progesterone is a steroid female hormone responsible for reproduction. In the second part of the menstrual cycle, the concentration of progesterone increases. The higher endogenous level of progesterone in pregnant women is said to increase the food intake and preserve energy for the baby. Some report suggests the combined use of progesterone with contraceptive pills during hormonal replacement leads to substantial weight gain. Thus, progesterone-induced hyperpiesia causes fat gain and is used as drug-induced obesity.[18] Pharmacological treatments such as the pancreatic lipase inhibitor orlistat have demonstrated promise in reducing the weight gain linked to progesterone-induced hyperphagia. Orlistat functions by preventing the breakdown of dietary fats into absorbable components by the pancreatic lipase enzyme. Orlistat lowers fat absorption by blocking lipase activity, thereby reducing total caloric intake and preventing excess fat from accumulating.

Furthermore, progesterone-induced hyperphagia has been countered by the use of drugs that alter serotonin reuptake, such as selective serotonin reuptake inhibitors or SSRIs. Serotonergic pathways play a crucial role in controlling appetite. These medications may help reduce overeating by supporting the restoration of the serotonin system. However, it has a number of adverse effects; thus, a polyherbal formulation is being used instead.

Results showed significant weight gain in the disease control group compared to the normal control groups using a 10 mg/kg dose of progesterone. This result shows the increase in food intake with progesterone-induced hyperphagia.

In this experimental model, progesterone produces hyperplasia *via* progestin receptors, which are expressed by serotonergic neurons. Some drugs like orlistat (30 mg/kg) suppress the progesterone-induced hyperpiesia by inhibiting the reuptake of 5HT serotonin, which regulates the food intake. The data represent that the total LDL, VLDL, triglyceride, glucose, and cholesterol levels are

raised in the disease control group compared to the treatment control group. The treatment control group with a higher dose is more effective than the lower dose, as it reduces the levels of total LDL, VLDL, triglycerides, glucose, and cholesterol while increasing the HDL level. Additionally, it lowers body weight and BMI.

These seven extracts appear in this activity because they regulate lipid metabolism by decreasing the activity of lipogenesis, inhibiting pancreatic lipase activity, and increasing fatty acid oxidation.

The study indicates that the above extract of the plant when prepared as a polyherbal formulation (syrup), exhibits an antiobesity effect by metabolizing lipids and interfering with the lipid metabolism mechanism. The potential for polyherbal formulations to modulate these pathways offers a promising natural approach to counteract obesity; further study is needed to quantify the formulation.

Conclusion

The formulation has the potential to control obesity. The effects of the formulation may include the suppression of fat deposition and lipid absorption, as well as hypolipidemic activity. The results confirmed that the formulation significantly reduced BMI, body weight, abdominal circumference, and fat deposition in tissue, as well as normalized lipid characteristics. This work can be expanded to identify the accountable compounds in the polyherbal formulation for its potent antiobesity effect.

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