RESEARCH ARTICLE

Development of Multi-particulate Formulation of Glabridin with L-Phenylalanine and Its Pre-clinical Evaluation for Anti-obesity Activity

Vyas UB*, Khobragade KS, Vyas PU

Datta Meghe College of Pharmacy, Sawangi (Meghe), Wardha, Maharashtra, India

Received: 11th August, 2023; Revised: 29th October, 2023; Accepted: 19th February, 2024; Available Online: 25th March, 2024

ABSTRACT

Obesity is a chronic condition that is defined by pathophysiological mechanisms that lead to an increase in adipose tissue mass as a result of positive energy balance. Obesity is a multi-factorial disorder involving different mechanisms of action like humoral and neurogenic actions. Hypertrophied and hyperplastic adipocytes are indicative of pathological obesity. In the current treatment regimen, the drugs used mainly focus on a single mechanism of action. Herbal medicines are reported to have anti-obesity activity but due to the presence of multiple phytoconstituents its very difficult for one to finalize the dose and issue of palatability. So the best solution is to focus on usage of active phytoconstituent as an anti-obesity medicine. A high protein diet proved to have anti-obesity activity, the main constituent responsible for this activity is L-phenylalanine which is an essential amino acid that produces its activity by stimulating the satiety centre and thus decreases food intake.

In this study, we are combining glabridin from Glycyrrhiza glabra and L-phenylalanine as both show anti-obesity activity through different action mechanisms, which may result in synergism. The study aims to prepare multi-particulate system (granules) of glabridin with L-phenylalanine and evaluate their anti-obesity and hypo-lipidemic action in HFD-treated rats.

Keywords: Multi particulate formulation, Glabridin, L-phenylaline, Anti-obesity activity.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.1.27

How to cite this article: Vyas UB, Khobragade KS, Vyas PU. Development of Multi-particulate Formulation of Glabridin with L-Phenylalanine and Its Pre-clinical Evaluation for Anti-obesity Activity. International Journal of Drug Delivery Technology. 2024;14(1):183-187.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Pathophysiological mechanisms that result in an increase in fat mass due to energy imbalance are the main symptoms of obesity, a chronic disorder. Obesity is caused by a combination of environmental (behavioral, social, cultural, and physiological) and genetic factors, which may function independently or together to contribute to the etiology of the disease.[1,2] obesity is generally due to energy imbalance–an excess of calories burned off relative to calories consumed.[3,4] This might be brought on by eating more calorie-dense, high-fat meals and engaging in less physical exercise due to the sedentary lifestyle, modes of transportation, and modern lifestyle.

Herbal medications are thought to work by suppressing appetite through various receptors acting centrally, blocking the absorption of triglycerides, increasing lipolysis, enhancing glycaemic control, promoting the differentiation of adipose tissue, and elevating energy expenditure and thermogenesis.[5,6] Their bioactive chemicals serve as the primary mediators of anti-obesity actions. These substances regulate inflammation and provide defense against oxidative damage. Glabridin from *Glycyrrhyza glabra* was reported to possess anti-obesity activity previously.[7,8]

High protein diet are proved to have satiety effect, thus showing anti-obesity activity. The main ingredient of a high-protein diet that shows this effect is L-phenylalanine. Being an essential amino acid, L-phenylalanine has to be taken externally.[9-11]

So, this study aims to combine these two active ingredients as they are proven to have anti-obesity activity but *via* different mechanisms. Combining these two may lead to enhancement of anti-obesity activity due to synergism.[12-14]

Even though many herbal drugs are effective, they lack off suitable dosage forms and result in problems with ease and accuracy of dosing of herbal drugs is one of the major factors that affect the overall acceptance of herbal drugs by patients as well as clinicians.[15-17] Formulating herbal active ingredients in dosage form, like granules, which are multi-particulate

*Author for Correspondence: vyasujwal@gmail.com

systems, can solve the problem of effectiveness and acceptance. And also solve the problem of dosing and palatability.[18-21]

MATERIALS AND METHODS

Materials

Glabridin, L-phenylalanine, starch, magnesium stearate, calcium phosphate diphasic, mannitol, citric acid, methyl paraben, propyl paraben, flavor, sodium saccharin, talc, color, PVP-K-30, DMSO.

Methods: Preparation of Multi Particulates (Granules) of Glabridin and L-Phenylalanine

Profile of the excipient

When starch absorbs water from bodily fluids, it swells and the granules break apart as a result. Mannitol and dibasic calcium phosphate were employed as bulking agents. Additionally, mannitol functions as a non-calorific sweetener and promotes quicker breakdown. Magnesium stearate aids in preventing fines development and granule attrition. The mixture of nontoxic, non-irritating methyl and propyl parabens keeps the product from breaking down. Citric acid helps to increase salivation so no need to take with water, which in turn causes the granules to disintegrate in the oral cavity. Therefore, patients who are on the go can use it conveniently. The flavoring ingredient and sodium saccharine somewhat mitigate the bitter taste of glabridin and L-phenylalanine.

Formulation

The wet granulation method used to make the granules. The ideal dosage combination is glabridin (10 mg) + L phenylalanine (100 mg). All ingredients are combined with citric acid in the right amounts, and the mixture is mashed in the appropriate container, and other ingredients are added. Then came the addition of the parabens, talc, starch, and dibasic calcium phosphate.

After adding enough distilled water to form a sticky dough-like mass, the mixture was passed through 22 no sieve to transform it into granules. The oven was used to dry the granules. Finally, magnesium stearate was added.

The final formulation, created in accordance with the table, contains 10% L-phenylalanine, 0.1% of glabridin and other excipients, as shown in Table 1

Evaluation

Different parameters will be checked as per procedure, such as rheological behavior, angle of repose, compression behavior and humidity absorption, which will be carried out for the characterization of prepared granules.

Angle of repose

After the cone was built using 5 gm of granules, the cone's height (h) and radius (r) were calculated. The final results were calculated by formula.

Tapped density

The mass of granules divided by the tapped volume is defined as the "tapped density." The volume that the mass of granules occupies after a measure is standardly tapped is known as the "tapped volume."

The formula for tapped density

Equation: $Dt = m / Vi$

In which case, m: Weight of mixture: 20 grams Vi: 40 mL tapped volume

A glass cylinder was taken for the test, and the volume was recorded after 50 taps.

Table 1: Formula for granules

S.N.	*Name*	*Nature*	*F1 (%)*	*F2 (%)*	*F3 (%)*	*F4(%)*
1	L-phenylalanine	API	10	10	10	10
2	Glabridin	API	0.1	0.1	0.1	0.1
3	Starch	Disintegrant	15	15	15	15
4	Magnesium stearate	Antiadherant	0.025	0.025	0.025	0.025
5	Calcium phosphate dibasic	Bulking agent	25	25	25	25
6	Mannnitol	Bulking agent	30	30	30	30
7	Citric acid	Taste masker, Sialagogue	11	11	11	11
8	Methyl paraben	Preservative	0.03	0.03	0.03	0.03
9	Propylparaben	Preservative	0.005	0.005	0.005	0.005
10	Raspberry flavor	Flavoring agent	0.0001	0.0001	0.0001	0.0001
11	Sodium saccharine	Sweetening agent	0.0149	0.0149	0.0149	0.0149
12	Talc	Sweetening agent	3.1	3.2	3.6	3.5
13	Color	Coloring agent	0.025	0.025	0.025	0.025
14	PVP-K-30	Binder	2.5	2.4	2.0	2.1
Total			100%	100%	100%	100%

Bulk density

It is the granule's bulk volume in proportion to its overall mass.

Equation: Db = m / Vo

In which case, m: 20 g is the blend's mass. Vo: Unplugged 45 mL of volume

A graded glass cylinder was utilized to conduct the test.

Loss on drying

To conduct loss on drying (LOD), a fixed quantity of the granules was dried at 105°C in the oven until a consistent weight was achieved.

The results for Angle of repose, Bulk density, tapped density and Loss on drying are enumerated in Tables 2 and 3.

The F1 formulation was chosen as the optimal formulation based on the results. The results of different parameters indicate the excellent flow qualities. Higher porosity is indicated by smaller tapped density and bulk values. The percentage LOD test values show a decreased moisture content in the formulation, which comply with the official limits.

Anti-Obesity Activity

Experimental animals

About 36 albino wistar rats, about 120 ± 10 g and roughly eight to ten weeks old, were acquired for this study from the CPRF, DMCOP, Sawangi Meghe, Wardha. In order to give the rats time to adjust, they were initially kept in standard cages with a standard light/dark cycle. Rat pellets from Vighnharta Food Maharashtra and tap water at will were the standard laboratory diet that the rats were fed during this period. The experimental protocols were approved by Datta Meghe College of Pharmacy's Institutional Animal Ethics Committee, located at DMIHER, Sawangi Meghe, Wardha.

Induction of obesity

For 12 weeks, the rats were fed a prepared HDF (as per formula) and water at will to develop obesity. With various adjustments, as indicated in Table 1. T1, the HFD diet's composition (g/kg diet) was created as per Table 4. Rats that were deemed obese and used in the study if only they have 310 or higher Lee Index, which is comparable to a human's body mass index.

Experimental protocol

Thirty-six rats were used in this study and were kept into six groups of six rats each at random. Normal (Sham) rats were kept in group I on a conventional pallet diet for a duration of 12 weeks. From the sixth to the 12th week, the rats in this group received no therapy other than 1% DMSO. Rats in group II (negative control) were fed an HFD for a duration of 12 weeks in order to create obesity. In addition, this group of rats received 1% DMSO from the sixth to the twelfth week of the study. Rats on a high-fat diet (HFD) were included in group III (positive control), where they received orlistat, the reference medication, from week six to week twelve. The rats in groups IV through VI (the experimental groups) were fed an HFD-induced diet and given either group IV glabridin (10 mg/kg body weight), group V L-phenylalanine (100 mg/kg body weight), or group VI glabridin + L-phenylalanine F1 formulation from the sixth to the twelfth week. Every treated rat was fed a high-fat meal for the duration of the dosing period. Throughout the course of the trial, water was provided at will to all of the experimental rats. Table 5 provides an overview of the experimental design.

Body weight and Lee index were measured every week by the Lee Index formula.

$$\text{Lee index (\%)} = \sqrt[3]{\frac{\text{Body Weight (g)}}{\text{Nose–to–Anus Length (cm)}}} \times 1000$$

Withdrawal of Blood

After an overnight fast, all of the animals were slain on the day of sacrifice by an excess dose of gaseous anesthetics in a glass vacuum desiccator. Using a 23G1 needle and a 5 mL syringe, cardiac puncture (lateral or ventral) was utilized to

Table 2: Results

Batch	*Angle of repose*	*Bulk density (gm/mL)*	*Tapped density (gm/mL)*	*% of LOD*
F1	21.14*	0.452	0.46	0.13
F2	22.25	0.436	0.47	0.16
F3	23.30	0.445	0.46	0.15
F4	23.49	0.426	0.45	0.14

Table 3: Results

S. N.	*Parameters*	*Observation*
1	Appearance	Solid
2	Color	Brown
3	Shape of granules	Spherical
4	Bulk density	0.452 gm/mL
5	Tapped density	0.46 gm/mL
6	Hausner ratio	1.13
7	Carrs compressibility index	12.1%
8	Angle of repose	21.14º
9	%LOD	0.13%

Table 4: Composition of high fat diet

Ingredients	*Diet (g/kg)*
Powdered NPD	375
Lard	290
Casein	265
Corn oil	10
Cholesterol	10
Vitamin and mineral mix	60
DI Methionine	03
Yeast powder	01
Sodium chloride	01

Table 5: Experimental design for *in-vivo* anti-obesity assays

Groups (5 rats/group)	*Treatments*	*Duration of obesity induction (weeks)*	*Duration of treatments (weeks)*
I (Normal control)	1% DMSO	No induction	No treatment
II (Negative control)	HFD+DMSO	6	No treatment
III (Positive control)	Orlistat+HFD	6	6
IV (Experimental group)	Glabridin (10 mg/kgbw) + HFD	6	6
V (Experimental group)	L-Phenylalanine (100 mg/kgbw) +HFD	6	6
VI (Experimental group)	Glabridin + L-Phenylalanine (F1) + HFD	6	6

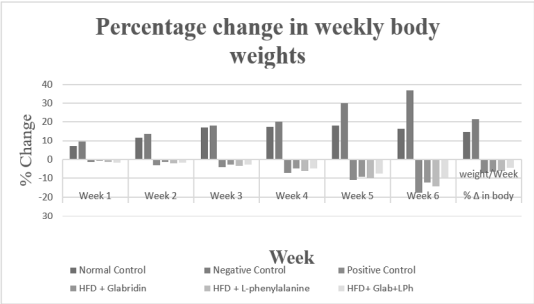

Figure 1: Effect of glabridin and L-phenylalanine on body weight

extract blood from each sacrificed rat's heart. Two vacutainers with well-marked labels were used to draw blood samples. The hematological parameters were computed using the first blood component. Blood from this sample was used to calculate the sugar level using a glucometer. To blood was allowed to stand for three hours to get it coagulated completely. The serum was produced by centrifuging blood at 3000 rpm for ten minutes. The serum was kept at -20°C until it was needed for the examination of biochemical markers and lipid profiles.

RESULTS

Effects of of Glabridin, L-Phenylalanine Individually and F1 (Glabridin+L-phenylalanine Granules) on Body Weights, Obesity and Lipid Profile of HFD- Induced Obese Rats

The normal control rat's weight gain rate in the first week of the trial differed significantly from their body weight gain, as shown by Figures 1 and 2. However, during the third, fourth, fifth, and sixth weeks of the study, there was no significant change in the weight of rats. During the study, the II group experienced a significant increase in the weight of rats. As a matter of fact, the rate of weight growth varied from 37.04 ± 4.03% in the sixth week to 09.80 ± 2.41% in the first. However, from the first to the sixth week of the trial, administration

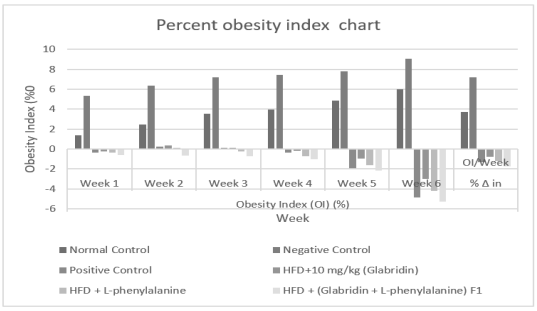

Figure 2: Effect of glabridin and L-phenylalanine on obesity index

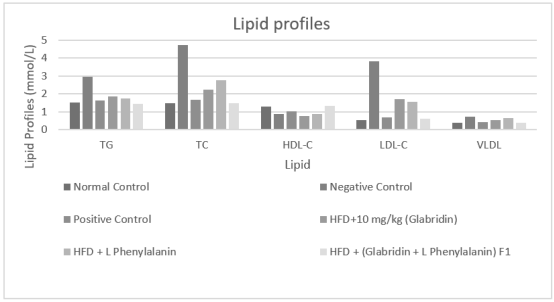

Figure 3: Effect of glabridin and L-phenylalanine on lipid profile

of the standard drug orlistat, causes a significant lowering of rat's body weight. The treatment group of glabridin and L-phenylalanine individually show a comparative decrease in body weight and obesity index but less as compared to standard drugs. The combination of glabrdin with L-phenylalanine (F1) granules formulation shows a markedly significant reduction in body weight as compared to the standard drug-treated group.

Figure 3 demonstrates a marked increase in all lipid markers, namely total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), and very low-density lipoprotein (VLDL) in 6th week in negative control group rats. Glabridin and L-phenylalanine reduce all lipid markers in rat's treated individually but not significantly more than the standard drug orlistat. But the formulation F1 (Glabridin + L-phenylalanine) significantly reduces all markers except HDL, which get a marked increase in level when compared to standard drugs.

CONCLUSION

Glabridin and L-phenylalanine show good and comparable anti-obesity activity when compared to standard drug orlistat by exerting significant change in body weight and obesity index. When HFD is used to create obesity in rats, the combination shows strong anti-obesity effects. When compared to a typical drug, it also dramatically raised HDL levels and markedly decreased lipid indicators such as total cholesterol, triglycerides, LDL, and VLDL.

REFERENCES

1. Ahirwar R, Mondal PR. Prevalence of obesity in India: A systematic review. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2019;13(1):318-21.
2. Borah AK, Sharma P, Singh A, Kalita KJ, Saha S, Borah

JC. Adipose and non-adipose perspectives of plant derived natural compounds for mitigation of obesity. Journal of Ethnopharmacology. 2021;280:114410.
3. Lim DW, Song M, Park J, Park SW, Kim NH, Gaire BP, et al. Anti-obesity effect of HT048, a herbal combination, in high fat diet-induced obese rats. Molecules. 2012;17(12):14765-77.
4. Cao X-j, Huang X-c, Wang X. Effectiveness of Chinese herbal medicine granules and traditional Chinese medicine–based psychotherapy for perimenopausal depression in Chinese women: a randomized controlled trial. Menopause. 2019;26(10):1193-203.
5. Zhang J, Wu X, Zhong B, Liao Q, Wang X, Xie Y, et al. Review on the diverse biological effects of glabridin. Drug Design, Development and Therapy. 2023:15-37.
6. Wang M, Zhang F, Zhou J, Gong K, Chen S, Zhu X, et al. Glabridin Ameliorates Alcohol-Caused Liver Damage by Reducing Oxidative Stress and Inflammation via p38 MAPK/Nrf2/NF-κB Pathway. Nutrients. 2023;15(9):2157.
7. Lee J-W, Choe SS, Jang H, Kim J, Jeong HW, Jo H, et al. AMPK activation with glabridin ameliorates adiposity and lipid dysregulation in obesity. Journal of lipid research. 2012;53(7):1277-86.
8. Choi LS, Jo IG, Kang KS, Im JH, Kim J, Kim J, et al. Discovery and pre-clinical efficacy of HSG4112, a synthetic structural analog of glabridin, for the treatment of obesity. International Journal of Obesity. 2021;45(1):130-42.
9. Kubota K, Mizukoshi T, Miyano H. A new approach for quantitative analysis of L-phenylalanine using a novel semi-sandwich immunometric assay. Anal Bioanal Chem. 2013;405(25):8093-103.
10. Weiser D, Bencze LC, Banoczi G, Ender F, Kiss R, Kokai E, et al. Phenylalanine Ammonia-Lyase-Catalyzed Deamination of an Acyclic Amino Acid: Enzyme Mechanistic Studies Aided by a Novel Microreactor Filled with Magnetic Nanoparticles. Chembiochem. 2015;16(16):2283-8.
11. Alamshah A, Spreckley E, Norton M, Kinsey-Jones J, Amin A, Ramgulam A, et al. L-phenylalanine modulates gut hormone release and glucose tolerance, and suppresses food intake through the calcium-sensing receptor in rodents. International Journal of Obesity. 2017;41(11):1693-701.
12. Malik ZA, Sharma PL. An ethanolic extract from licorice (glycyrrhiza glabra) exhibits anti-obesity effects by decreasing dietary fat absorption in a high fat diet-induced obesity rat model. International Journal of Pharmaceutical Sciences and Research. 2011;2(11):3010.
13. Ahn J, Lee H, Jang J, Kim S, Ha T. Anti-obesity effects of glabridin-rich supercritical carbon dioxide extract of licorice in high-fat-fed obese mice. Food and chemical toxicology. 2013;51:439-45.
14. Norton M. The effects of high protein diets and L-Phenylalanine on energy and glucose homeostasis. 2019.
15. Wang Y, Liu Y, Lv Q, Zheng D, Zhou L, Ouyang W, et al. Effect and safety of Chinese herbal medicine granules in patients with severe coronavirus disease 2019 in Wuhan, China: a retrospective, single-center study with propensity score matching. Phytomedicine. 2021;85:153404.
16. Yang X, Wang S, Qi L, Chen S, Du K, Shang Y, et al. An efficient method for qualitation and quantitation of multi-components of the herbal medicine Qingjin Yiqi Granules. Journal of Pharmaceutical and Biomedical Analysis. 2023;227:115288.
17. Shen Z, Zheng K, Zou J, Gu P, Xing J, Zhang L, et al. Chinese herbal extract granules combined with 5-aminosalicylic acid for patients with moderately active ulcerative colitis: study protocol for a multicenter randomized double-blind placebo-controlled trial. Trials. 2021;22:1-9.
18. Kandale J., Sangashetty J., Mahaparale P., Phytochemical Evaluation of Extracts and Pharmacological Activity of Polyherbal Emulgel. IJPQA. 2023;2: 341-345.
19. Rani L., Sharma N., Singh S., Grewal A.S. Therapeutic potential of vitamin c: An overview of various biological activities. Int. J. Pharm. Qual. Assur. 2019;10:605–612.
20. Mehata A., Jain N., Grobler A. Role of Novel Drug Delivery Systems in Bioavailability Enhancement: At A Glance. Int. J. Pharm. Drug Del. Tech. 2016;6(1):7-26.
21. Khulood S. The Effect of Oral Administration of Green Tea and Ginger Extracts on Blood Glucose in Diabetic Rats. Int. J. Pharm. Drug Del. Tech. 2019;9(3):418-423