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Effects of a Novel Botanical Beverage on Metabolic Parameters and Body Composition

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Abstract: Obesity and its complications require new interventions. This study evaluates GLY.PLANT-HORMO AMPOULES, a novel botanical beverage with 18 bioactive ingredients extracted using advanced technologies, for effects on metabolic parameters, body composition, and skin collagen. A 12-week randomized, double-blind, placebo-controlled trial was conducted in 136 adults with Class I obesity (BMI 25–30 kg/m ²). Parallel experiments in db/db mice assessed dose-dependent metabolic effects. In humans, the beverage significantly reduced body weight (−4.31 kg), body fat (−5.02%), and waist/hip/thigh circumferences ($P < 0.05$), with sustained effects observed even 4 weeks after supplementation. Participants also reported improved metabolism (97.01%), reduced skin laxity (98.51%) and reduced waist circumference (100%). In db/db mice, high-dose treatment was more effective than the metformin group in lowering fasting glucose, triglycerides, and cholesterol. Notably, the formulation upregulated key metabolic regulators, including UCP-1 (8.21-fold), AMPK (7.02-fold), irisinc (9.14-fold), and TCA cycle enzymes(4.21-fold). Both collagen (62%) and elastin (52%) synthesis significantly increased. GLY.PLANT-HORMO AMPOULES synergistically improves metabolic health and skin tightness via multi-targeted mechanisms, offering a promising strategy for metabolic syndrome management.

1. Introduction

Obesity, a global epidemic affecting over 650 million adults, is a major driver of metabolic dysfunction, including insulin resistance and increased risk of type 2 diabetes and cardiovascular diseases. While pharmacological interventions have limitations, natural botanical compounds offer promising alternatives due to multi-targeted mechanisms and safety. For example, white mulberry leaf inhibits α-glucosidase[1], and chromium enhances insulin sensitivity[2]. Emerging evidence also links metabolic health and skin tightness.

This study focuses on GLY.PLANT-HORMO AMPOULES (REALM NUTRITION), a novel formulation integrating 18 bioactive ingredients, including GLP-MODEX™ and TCAthin™. A key innovation is the sequential Ultrasound-Assisted Extraction (UAE) and Pulsed Electric Field (PEF)

treatment used in its production. This combined approach increases the yield of phytochemicals by 3- to 5-fold, as demonstrated in a study on betaine extraction[3].

This study investigates whether GLY.PLANT-HORMO AMPOULES improves body composition, metabolic parameters, and skin collagen synthesis in db/db mice, a model of obesity and diabetes. Our findings aim to advance the development of evidence-based, multi-functional interventions for metabolic syndrome.

2. Method

2.1 Test Beverages

Test beverages were prepared in 15 mL aliquots. The active beverage, GLY.PLANT-HORMO AMPOULES (REALM NUTRITION), contained 18 ingredients, including GLP-MODEX™ (White Mulberry Leaf, Chromium, Black Tea, Purslane, and Holy Basil Leaf), TCAthin™ (Acacia Gum, Bergamot, Psyllium Husk, Mate Tea, and Guarana Seed), Green Cardamom, Hibiscus, Chlorella, and other beneficial ingredients. The placebo beverage, lacking active ingredients, served as a control. A monitor confirmed no discernible differences in appearance or taste between the active and placebo beverages before dispensing.

2.2 Raw Material Processing

Raw materials underwent sequential Ultrasound-Assisted Extraction (UAE) and Pulsed Electric Field (PEF) treatment in pure water. UAE was performed at 20 kHz and 100W for 30 minutes (5s on/2s off pulse)[4]. The resulting extract was immediately processed via PEF, applying 1 kV/cm pulses at 10 Hz (100 μs pulse width) for 5 minutes. A circulating chiller maintained the extraction temperature below 10 ℃[5].

2.3 Human Study

2.3.1 Subjects

Subjects were females aged 20 to below 65 years with a BMI of 25 to below 30 kg/m^2. Exclusion criteria included being on a diet; use of medications or supplements affecting body fat or lipid metabolism; excessive alcohol consumption; current treatment for any disease; potential allergy symptoms; history of serious diseases (e.g., liver, kidney, heart, thyroid, adrenal, metabolic disorders); severe anemia; or history of digestive disease.

2.3.2 Study Design

A randomized, double-blind, placebo-controlled parallel group study was conducted over 12 weeks, including an 8-week test beverage ingestion period (0 to 8 w) and a 4-week follow-up (8 to 12 w). Subjects were screened for eligibility over 2 weeks. Visits occurred at 0, 4, 8, and 12 w for interviews and anthropometric parameter measurements. At screening, subjects completed a lifestyle questionnaire, and female subjects took a pregnancy test.

Subjects were randomly assigned (1:1) to groups, with the assignment list stored securely until database lock. All study personnel (except the controller) and subjects remained blinded throughout

the study. Subjects consumed the test beverage once per day, ideally 30 minutes after breakfast, but on test days, they ingested it after testing. They were instructed to maintain usual habits and avoid over-drinking. Oral medications/supplements affecting body fat or lipid metabolism were prohibited. Alcohol was prohibited the day before testing, and the evening meal had to be finished by 22:00, with no eating or drinking (except water) until testing completion the following day[6].

2.3.3 Measurement of Obesity Parameters

Height was measured at screening. Bodyweight, body fat ratio (by BIA using an Inner Scan 50V BC-621), waist circumference, hip circumference, and thigh circumference were measured at each visit. BMI was calculated from height and body weight.

2.3.4 Subject Questionnaire

Subjects completed a self-assessment questionnaire at 4, 8, and 12 weeks, rating their agreement on a 5-point scale. The percentage of subjects scoring 4 or 5 (agreement/relative agreement) was calculated for each question.

2.4 Animal Study

2.4.1 Animal Experiments

C57BLKS/JGpt db/db male mice (31−35 g, 6 weeks old) were purchased. Mice were housed under controlled conditions with ad libitum access to food and water. Db/db mice were fed a high-fat diet (60% fat) and divided into four groups (n = 10/group): Control (vehicle), L-dose (1500 mg/kg test substance), H-dose (3000 mg/kg test substance), and Metformin (200 mg/kg). Non-diabetic control M/m mice were fed a standard diet and served as a vehicle group (n = 10). All groups were treated for 8 weeks. Body weight, fasting blood glucose, food intake, and water consumption were monitored weekly[7].

2.4.2 Assessment of Glucose Metabolism and Insulin Sensitivity

Fasting Blood Glucose (FBG): Measured weekly after a 6-hour fast using a Contour Plus glucometer. Oral Glucose Tolerance Test (OGTT): Performed at week 8 after a 6-hour fast. Mice received glucose (2 $g \cdot kg^{-1}$) orally, and blood glucose was measured at 0, 30, 60, 90, and 120 minutes using a Contour Plus glucometer[7].

2.4.3 Assessment of Lipid Metabolism

Lipid Profile: Serum triglycerides (TG) and total cholesterol (TC) were measured using enzymatic assay kits (Abcam, ab65336 and ab65390, respectively). Liver Triglyceride Content: Liver tissue was homogenized in RIPA buffer, and triglyceride content was measured using an enzymatic assay kit (Abcam, ab65336)[7].

2.4.4 Assessment of Energy Metabolism & Mitochondrial Function

UCP-1 and AMPK Expression: Adipose tissue was analyzed by Western blot using anti-UCP-1

(Abcam, ab10983, 1:1000) and anti-AMPK (Cell Signaling, 2532, 1:1000) antibodies.Irisin Levels: Serum irisin levels were measured using an ELISA kit (Phoenix Pharmaceuticals, EK-067-29). TCA Cycle Enzyme Activity: Liver tissue homogenates were subjected to Western blot analysis to measure Citrate Synthase (CS) expression using an anti-CS antibody (Abcam, ab129095, 1:1000)[8].

2.4.5 Assessment of Skin Collagen Synthesis

Skin samples were fixed in 4% paraformaldehyde, sectioned at 5 μm, and stained with Masson's trichrome and antibodies against collagen type I (Abcam, ab34710, 1:500) and elastin (Abcam, ab21610, 1:500). Images were captured using a Leica DMi8 microscope[9].

2.5 Statistical Analysis

Data were expressed as means (SEM). Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Differences were considered significant at $P < 0.05$. All statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA).

3. Results

3.1 Human Study

As shown in Table 1, there is little difference in nutritional composition between GLY.PLANT-HORMO AMPOULES (active group) and the placebo beverage. Additionally, Table 2 indicates that the age and BMI of participants in both groups are comparable. Supplementation with GLY.PLANT-HORMO AMPOULES significantly improved body composition over the 12-week trial (Table 3). During the 8-week intervention, the active group exhibited progressive reductions in body weight (−4.13 ± 0.38 kg), body fat ratio (−4.87 ± 0.32%), waist circumference (−9.92 ± 1.82 cm), hip circumference (−7.72 ± 1.62 cm), and thigh circumference (−8.02 ± 1.82 cm) compared to baseline and placebo ($P < 0.05$). Notably, these improvements persisted during the 4-week follow-up period, with no rebound effect observed in any parameter: body weight remained reduced by −4.31 ± 0.78 kg, body fat ratio by −5.02 ± 0.72%, waist circumference by −10.12 ± 1.72 cm, hip circumference by −7.81 ± 1.46 cm, and thigh circumference by −8.12 ± 1.71 cm ($P < 0.01$ vs. Placebo, $P < 0.01$ vs. 0 w). In contrast, the placebo group showed no significant changes, with slight increases in body weight (+0.35 ± 0.88 kg) and body fat percentage (+0.38 ± 0.78%) by week 12.

The GLY.PLANT-HORMO AMPOULES showed excellent tolerability, with 100% of Group A reporting no adverse reactions during the 8-week intervention (Table 4). Most participants (95.52%) experienced improved metabolism, 91.04% noted appetite control, and all (100%) reported sustained waist reductions. Skin laxity benefits increased from 91.04% at week 4 to 97.01% by week 8, and 100% reported a more defined body contour at week 8. These benefits, including metabolic enhancement (97.01% at week 12), waist reduction (100%), and skin laxity improvement (98.51%), persisted or improved during the 4-week follow-up. The control group without active ingredients showed no significant improvements.

Table 1 Nutritional compositions of test beverages (per 100 mL)

	Active beverage	Placebo beverage
Energy (kcal)	2	1.9
Protein (g)	0.1	0.1
Lipid (g)	ND	ND
Available carbohydrate (g)	ND	ND

ND, not detectable

Table 2 Baseline characteristics of the subjects

Parameter	Active group	Placebo group
n	67	69
Age (years)	44.8 (1.2)	44.8 (1.2)
BMI (kg/m^2)	27.56 (0.34)	27.20 (0.33)

No significant differences were observed in any parameters between the two groups

Table 3 Anthropometric Changes

Parameter	Group	Baseline 0w	Change from the value at 0 w		
			Ingestion Period		Follow-up period
			4 w	8 w	12 w
Body weigh (kg)	A	77.65 (0.88)	-2.61 (0.21)$^{\#*}$	- 4.13 (0.38)$^{\#\#**}$	-4.31 (0.78) $^{\#\#**}$
	p	78.42 (0.87)	+0.25 (0.41)	+0.14 (0.71)	+0.35 (0.88)
Body fat ratio (%)	A	39.12 (0.79)	-3.12 (0.17)$^{\#*}$	- 4.87 (0.32)$^{\#*}$	-5.02 (0.72)$^{\#\#**}$
	P	40.15 (0.78)	+0.28 (0.49)	+0.13 (0.51)	+0.38 (0.78)
Waist circumference (cm)	A	88.15 (0.55)	-6.11 (1.12)$^{\#\#**}$	- 9.92 (1.82)$^{\#\#**}$	-10.12 (1.72)$^{\#\#**}$
	P	87.19 (0.52)	+0.12 (0.02)	+0.01 (0.01)	+0.28 (0.08)
Hip circumference (cm)	A	105.59 (0.45)	-3.92 (1.22)$^{\#*}$	- 7.72 (1.62)$^{\#\#**}$	-7.81 (1.46)$^{\#\#**}$
	P	106.08 (0.40)	+0.01 (0.02)	-0.01 (0.03)	+0.03 (0.02)
Thigh circumference (cm)	A	67.59 (0.85)	-4.11 (1.52)$^{\#*}$	- 8.02 (1.82)$^{\#\#**}$	-8.12 (1.71)$^{\#\#**}$
	P	68.1 (0.91)	+0.02 (0.02)	+0.01 (0.01)	+0.08 (0.06)

A, Active group (n = 69); P, Placebo group (n =67).

$^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. Placebo group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 0 w.

Table 4 Self-assessment

Question	Group	Ingestion Period		Follow-up period
		4 w	8 w	12 w
Improve body metabolism	A	89.55%	95.52%	97.01%
	p	7.25%	4.35%	4.35%
Reduce waist circumference	A	97.01%	100%	100%
	P	0%	1.45%	0%
Reduce skin laxity	A	91.04%	97.01%	98.51%
	p	0%	1.45%	0%
The product is gentle	A	100%	100%	^a^
	P	100%	99%	^a^
Feel more curvy	A	98.51%	100%	98.51%
	P	0%	1.45%	0%
Decreased desire to eat	A	89.55%	91.04%	74.63%
	P	0%	1.45%	0%

^a^ Not assessed at week 12 (post-supplementation phase).

3.2 Animal Study

In db/db mice, GLY.PLANT-HORMO AMPOULES exhibited dose-dependent efficacy in mitigating obesity and metabolic dysfunction (Tables 5–9). High-dose (H-dose) treatment reduced body weight by 12.52% (−5.66 g vs. control, $P < 0.01$) as shown in Table 5, with an accompanying 8.51% (−2.15 kcal/day vs. control, $P < 0.01$) reduction in food intake. The treatment also significantly lowered fasting blood glucose (−69.78 mg/dL, $P < 0.01$) and outperformed metformin group in reducing OGTT AUC (−12,966.67 vs. −10,999.45; $P < 0.01$), as detailed in Table 6.

Lipid metabolism improved markedly: high-dose treatment reduced serum triglycerides by 22.06% (−39.78 mg/dL, $P < 0.01$), serum total cholesterol by 18.24% (−40.22 mg/dL, $P < 0.01$), and liver triglycerides by 23.45% (−39.78 mg/dL, $P < 0.01$), surpassing the effects of metformin (Table 7). Mechanistic analyses revealed upregulated UCP-1 (8.21-fold vs. control, $P < 0.01$) and AMPK expression (7.02-fold, $P < 0.01$), alongside elevated irisin levels (9.14-fold, $P < 0.01$) and citrate synthase activity (4.21-fold, $P < 0.01$), indicating enhanced mitochondrial thermogenesis and fatty acid oxidation (Table 8).

Strikingly, H-dose increased skin collagen type I and elastin synthesis by 62% and 52%, respectively ($P < 0.01$; Table 9), aligning with human self-reports of skin benefits. Non-diabetic M/m mice showed no significant changes, confirming the formulation's targeted action in metabolic dysfunction.

Table 5 Body Weight, Food Intake, and Water Consumption

Group	Body Weight (g)	Food Intake (kcal/day)	Water Consumption (mL/day)
Control (db/db)	45.22(2.11)	25.26(1.31)	6.53(0.41)
L-dose (db/db)	42.31(1.82)*	24.16 (1.22)*	6.44(0.36)
H-dose (db/db)	39.56 (1.54)**	23.11(1.24)**	6.32(0.35)
Metformin (db/db)	40.11(1.73)**	23.59(1.36)*	6.26(0.41)
M/m (Control)	28.42(1.28)	11.29(1.02)	5.49(0.21)

Data are expressed as means (SEM); *p < 0.05, **p < 0.01 vs. Control (db/db).

Table 6 Glucose Metabolism

Group	Fasting Blood Glucose (mg/dL)	AUC (OGTT)
Control (db/db)	320.45 (15.23)	45012.34 (2501.12)
L-dose (db/db)	280.12 (12.45)*	38023.56 (2001.34)*
H-dose (db/db)	250.67 (10.23)**	32045.67 (1801.56)**
Metformin (db/db)	260.34 (11.56)**	34012.89 (1901.23)**
M/m (Control)	110.23 (5.12)	15023.45 (1001.34)

AUC: Area under the curve for OGTT; *p < 0.05, **p < 0.01 vs. Control (db/db).

Table 7 Lipid Metabolism

Group	Serum TG (mg/dL)	Serum TC (mg/dL)	Liver TG (mg/g tissue)
Control (db/db)	180.34 (10.12)	220.45 (12.34)	50.23 (3.12)
L-dose (db/db)	160.12 (8.23)*	200.34 (10.12)*	45.12 (2.34)*
H-dose (db/db)	140.56 (7.34)**	180.23 (9.45)**	38.45 (2.12)**
Metformin (db/db)	161.34 (8.12)*	199.12 (10.23)*	46.23 (2.45)**
M/m (Control)	80.12 (5.23)	120.34 (6.12)	20.12 (1.23)

TG: Triglycerides; TC: Total cholesterol; *p < 0.05, **p < 0.01 vs. Control (db/db).

Table 8 Energy Metabolism & Mitochondrial Function

Group	UCP-1 Expression (Relative)	AMPK Expression (Relative)	Change in Irisin (ng/mL)	Citrate Synthase Expression (Relative)
Control (db/db)	1.00 (0.12)	1.00 (0.11)	+1.01 (0.81, 1.12)	1.00 (0.11)
L-dose (db/db)	4.52 (0.21)**	3.42 (0.23)**	+6.03 (5.80, 6.20)**	2.21 (0.25)*
H-dose (db/db)	8.21 (0.32)**	7.02 (0.31)**	+9.23 (8.82, 9.31)**	4.21 (0.36)**
Metformin (db/db)	1.83 (0.22)	1.62 (0.24)	+2.51 (2.31, 2.71)*	1.72 (0.14)
M/m (Control)	2.12 (0.31)	2.23 (0.32)	+1.51 (1.34, 1.71)	1.23 (0.32)

Data are presented as mean (SEM) for UCP-1, AMPK and Citrate Synthase, and mean changes (95% CI) for irisin; *p < 0.05, **p < 0.01 vs. Control (db/db).

Table 9 Skin Collagen Synthesis

Group	Collagen Type I (Relative)	Elastin (Relative)
Control (db/db)	1.00 (0.11)	1.00 (0.12)
L-dose (db/db)	1.32 (0.21)*	1.23 (0.11)*
H-dose (db/db)	1.62 (0.22)**	1.52 (0.21)**
Metformin (db/db)	1.13 (0.23)	1.02 (0.22)
M/m (Control)	0.87 (0.31)	0.92 (0.23)

Data are normalized to Control (db/db); *p < 0.05, **p < 0.01 vs. Control (db/db).

4. Discussion

This study demonstrates that GLY.PLANT-HORMO AMPOULES synergistically improves metabolic health, body composition, and skin tightness. The observed weight loss (−4.31 kg, $P < 0.01$) and body fat reduction (−5.02%, $P < 0.01$) in humans surpass typical nutraceutical outcomes for Class I obesity, likely due to the combined action of GLP-MODEX™ (e.g., mulberry leaf and chromium enhancing insulin sensitivity[1,2]) and TCAthin™ (e.g., mate tea promoting TCA cycle efficiency[10]). In animal models, GLY.PLANT-HORMO AMPOULES treatment demonstrated significant improvements in blood glucose, cholesterol, and triglycerides, indicating its efficacy in promoting weight loss and enhancing metabolic health, further reinforcing the findings from the human trials.

Mechanistically, UCP-1 upregulation (8.21-fold, P < 0.01) indicates white adipose tissue (WAT) browning, a key driver of thermogenesis, while AMPK activation (7.02-fold, $P < 0.01$) enhances metabolic flux via fatty acid oxidation and glucose uptake[8]. The formulation significantly increased irisin (9.14-fold, $P < 0.01$), a myokine linked to exercise-induced weight loss. Since

maintaining elevated irisin in obese individuals typically requires sustained exercise (e.g., 24 weeks, 3 times/week[11]), this increase suggests a potential mechanism by which the formulation promotes weight loss, potentially mimicking some benefits of exercise[12]. Concurrently, citrate synthase upregulation (4.21-fold, $P < 0.01$) reflects accelerated TCA cycle activity, critical for mitochondrial energy production and complete metabolism of sugars and fats.

Additionally, appetite control was noted by 91.04% of participants, supporting the formulation's efficacy in managing hunger. In animal models, a corresponding 8.51% reduction in food intake (−2.15 kcal/day vs. control, $P < 0.01$) further underscores the formulation's role in appetite regulation, which may be linked to appetite-related hormonal changes associated with enhanced metabolic function and body composition improvement[8].

The novel enhancement of skin collagen (62%, $P < 0.01$) and elastin synthesis (52%, $P < 0.01$) may result from the grape and gardenia extracts in the formulation, which are reported to stimulate collagen synthesis via TGF-β pathways[13-14]. This aligns with human self-reports of reduced skin laxity (98.51% agreement).

5. Conclusion

GLY.PLANT-HORMO AMPOULES represents a breakthrough in nutraceutical science, integrating metabolic regulation with dermatological benefits. Its multi-targeted action—enhancing insulin sensitivity, mitochondrial function, and collagen synthesis—positions it as a holistic intervention for metabolic syndrome. Future studies should prioritize dose optimization, mechanistic validation in humans, and investigations into synergistic interactions among its 18 components.

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