

## Effect of the Several Herbal Medicines Mixture on BMI and Lipid Profile -The Animal Study

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### Abstract

**Objective:** Obesity involves complex pathological mechanisms. Multi-herbal formulations targeting diverse pathways may provide synergistic therapeutic benefits. This study aimed to evaluate the anti-obesity effects of a standardized multi-herbal formulation (GUTAC), consisting of Glycyrrhiza glabra, Urtica dioica, Trigonella foenum-graecum, Artemisia persica and Camellia sinensis.

**Materials and Methods:** Obesity was induced in male Wistar rats (n= 30) via a high-fat diet (HFD) for three months. Subsequently, the rats were divided into three groups (n= 10 per group): (1) HFD alone, (2) HFD with GUTAC, and (3) standard diet (SD) with GUTAC, for an additional three months. Key parameters such as body mass index (BMI), blood biomarkers, and histopathological changes in the liver and kidney were evaluated.

**Results:** BMI was significantly lower in both the HFD+GUTAC and SD+GUTAC groups compared to the HFD group ( $P < 0.05$ ). Notably, the SD+GUTAC group exhibited a more pronounced BMI reduction, indicating that combining GUTAC with a standard diet yields greater benefits than its combination with a high-fat diet. GUTAC treatment significantly reduced blood glucose levels ( $P = 0.0013$ ), liver enzyme activity (AST and ALT), and improved lipid profiles, including total cholesterol, HDL, LDL, and triglycerides, compared to the HFD group (all  $P < 0.05$ ). Furthermore, GUTAC enhanced renal function markers (BUN and creatinine) and mitigated hepatic steatosis, as evidenced by histological analysis.

**Conclusion:** The standardized multi-herbal GUTAC formulation demonstrated significant anti-obesity, hypolipidemic, hepatoprotective, and renoprotective effects in a rat model. These benefits are likely mediated by the bioactive compounds' modulation of lipid metabolism, oxidative stress, and inflammatory pathways. These findings highlight GUTAC's potential as a multi-target therapeutic strategy for obesity, warranting further exploration in human clinical trials.

**Keywords:** obesity, herbal medicine, complementary therapies, lipid profile, herbs

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## Introduction

Obesity is a complex, multifactorial nutritional and metabolic disorder characterized by excessive accumulation of adipose tissue due to a chronic imbalance between caloric intake and energy expenditure (1,2). This chronic condition represents one of the most rapidly escalating global health burdens of the 21st century. Alarming, it is estimated that over 1.9 billion adults aged 18 years and above are overweight, with 11% of men and 15% of women classified as obese worldwide, according to the latest figures from the World Health Organization (3).

The pathophysiology of obesity and dysregulation of body weight and energy homeostasis involves an intricate interplay of various physiological mechanisms. At the molecular level, aberrant insulin metabolism and signaling pathways (4), alterations in gut microbiome composition and function (5), dysregulated appetite and satiety cues (6), impaired adipocyte differentiation and lipid metabolism (7), disruptions in hypothalamic-dependent neuroendocrine circuits (8), and leptin hormone resistance (9) have been implicated as key contributing factors.

In the quest for effective interventions, numerous studies have explored the therapeutic potential of herbal medicine as an adjunctive approach for weight management and obesity treatment. Notably, species from the genus *Artemisia* have exhibited promising anti-obesity effects, primarily mediated through modulation of adipocyte metabolism and adipogenesis (10). Licorice root (*Glycyrrhiza* spp.), rich in bioactive compounds like glycyrrhizin, has been shown to suppress weight gain, potentially by modulating the composition and metabolic activity of the gut microbiome (11). *Urtica dioica* (stinging nettle) has garnered attention for its anti-diabetic properties, attributed to its ability to stimulate insulin secretion and enhance glucose uptake (12). *Camelia sinensis* (green tea), abundant in polyphenolic

compounds like epigallocatechin gallate (EGCG), may contribute to weight management by decreasing the absorption of lipids and proteins in the gastrointestinal tract (13). Additionally, *Trigonella foenum-graecum* (fenugreek) seeds have been reported to reduce appetite and food intake, thereby inhibiting weight gain (14).

While most previous studies have focused on individual plants and targeted specific mechanisms involved in obesity pathogenesis, the rationale behind this study is to employ a multi-targeted approach by utilizing a mixture of known herbs. These selected herbs have been reported to primarily modulate distinct mechanisms implicated in obesity development and progression. To achieve this, we formulated a novel herbal mixture comprising five herbs: *Glycyrrhiza glandulifera*, *Urtica dioica*, *Trigonella foenum-graecum*, *Artemisia persica*, and *Camelia sinensis* (hereafter referred to as the GUTAC mixture). By combining these herbs, we hypothesize that the GUTAC mixture may exert complementary effects, simultaneously targeting multiple pathways involved in obesity pathogenesis, thereby offering a more comprehensive and potentially effective approach to weight management (15).

## Materials and methods

Thirty male Wistar rats (weight: 250-300g, age: 2-3 month) were obtained from the animal housing facility of Rafsanjan University of Medical Sciences, Rafsanjan, Iran. They were housed under controlled conditions at a temperature of 23°C with a 12-hour light/dark cycle (four rats per cage). The animals had free access to water.

Initially, thirty rats were fed a high-fat diet (HFD) for three months to induce obesity (16). The obesity model was considered successful when the body weight increase to more than 20% (compared to rats fed with standard diet: SD). The HFD provided 60% fat, 25% protein, and 17% carbohydrate and was purchased

from the University of Isfahan (17). The animal weights were recorded each month using a digital balance scale throughout the entire experiment. (18). It should be noted that, for simplicity, the data for the establishment of obesity is not presented.

After confirmation obesity: the animals were divided into three groups, each containing 10 rats, the first group served as the control group and receiving the HFD. In the second group, the HFD was supplemented with GUTAC. In the third group, the HFD was replaced with a standard laboratory rodent diet (SD), and GUTAC was added to the treatment protocol. The treatment protocols were continued for three months in all groups.

### **Preparation of the GUTAC (Glycyrrhiza glabra, Urtica dioica, Trigonella foenum-graecum, Artemisia persica and Camellia sinensis)**

*Trigonella foenum-graecum*: One kilogram of fenugreek seed powder was boiled in 10 liters of distilled water for 30 minutes, cooled at room temperature for 30 minutes, filtered twice, and the residue was evaporated at 35°C. Finally, 190 grams of dry powder extract was obtained, corresponding to a yield of 13 grams of dry extract per 300 grams of dried fenugreek powder (19).

*Camellia sinensis*: Ground green tea leaves were used for extraction. The leaves were placed in boiling water (97°C) at a ratio of 1:10 (plant material: water) for 20 minutes. The solution was filtered, and this process was repeated three times. The combined filtrate was then passed through a 0.45-micron filter paper. To the half volume of the filtrate, an equal volume of ethyl acetate was added. After waiting for 15 minutes, the temperature was raised to 70°C until the ethyl acetate evaporated, leaving behind the concentrated extract (20).

*Glycyrrhiza glabra*: Licorice root was ground and placed in distilled water at 90°C. The mixture was then poured into ammonium water and stirred. Finally, the solution was

filtered and lyophilized (freeze-dried) to obtain the dry extract. The yield of dry extract was 3 grams per 90 grams of dry licorice powder (21).

*Artemisia persica*: One gram of ground *Artemisia* leaf was mixed with 10 ml of 95% ethanol. The mixture was kept in the dark at room temperature (36°C) for 24 hours. The mixture was then filtered, and the filtrate was dried at 45°C to obtain the dry extract. The yield of dry extract was 18 grams per 300 grams of dried *Artemisia* powder (22).

*Urtica dioica*: For extraction, one kilogram of nettle leaves was placed in 50% ethanol for 2 hours. The leaves were then separated, and the solution was concentrated using a rotary evaporator and finally dried at 45°C. The yield of dry extract was 85 grams per 300 grams of dry nettle powder (23).

The GUTAC mixture is standardized based on a minimum content of 0.08% chlorogenic acid by HPLC analysis.

### **Serum Biochemical Assessments**

Blood samples were collected in fasting rats from the retro-orbital plexus under anesthesia. Samples were obtained at 3 time points during the study (before the start of experiment, middle of experiment -45days- and after complementation of the study -90days-). Serum was separated by centrifugation at 3,000 ×g at 4°C for 20 minutes and stored at -80°C for later measurements. Serum levels of triglycerides (TGs, cat no: 1920524), total cholesterol (TC, cat no: 1630724), high-density lipoprotein cholesterol (HDL-C, cat no: 1751923), low-density lipoprotein cholesterol (LDL-C, cat no: 1831123), and fasting glucose (cat no: 1740724) were measured enzymatically (Spectrum; GmbH, Hannover, Germany) according to the manufacturer's instructions (Delta Darman Part company). Additionally, the activities of alanine aminotransferase (ALT, cat no: 1540524), aspartate aminotransferase (AST, cat no: 1590524), and alkaline phosphatase (ALP, cat no: 1530724) were measured using

an auto-analyzer (BT3000). Serum concentrations of creatinine (Cr, cat no: 1711224) and blood urea nitrogen (BUN, cat no: 1932523) were also measured to assess kidney function. All biochemical measurements were done using Delta Darman Part company kits.

Pathological examinations of rat tissues were performed using hematoxylin and eosin (H&E) staining. The liver and kidney were excised using a surgical scissor and placed in 10% formaldehyde buffer for fixation. Kidney sections were assessed for glomerular atrophy, inflammatory cell infiltration, and tubular necrosis. Liver sections were evaluated for congestion, steatosis, and pyknosis (nuclear condensation). The histopathological assessments were performed by a pathologist who was blinded to the study groups. Tissue analysis was conducted using a light microscope (Nikon Labophot, Japan). Steatosis in the liver was quantified by scoring from 0 to 3 (0= normal, no damage observed; 1= minor damage; 2= moderate damage; 3= severe injury).

### Statistical analysis

All parameters were analyzed using SPSS software (version 16). Data are presented as the mean  $\pm$  standard error of the mean (SEM). Comparisons between groups and time points were performed using two-way repeated measures analysis of variance followed by Tukey's multiple comparison test. All p-values were two-tailed, and the alpha level of significance was set at 0.05.

### Ethical considerations

All experimental procedures in this study were approved by the Ethical Committee of Rafsanjan University of Medical Sciences (Ethical code: IR.RUMS.REC.1399.071) and conducted in accordance with the United States NIH Guide for the Care and Use of Laboratory Animals (publication no. 85-23).

## Results

### BMI

The two-way repeated measures ANOVA revealed a significant main effect of group ( $P=0.0002$ ), indicating that BMI differed significantly among the three groups (HFD, HFD+GUTAC, and SD+GUTAC). There was also a significant main effect of time ( $P=0.001$ ), suggesting that BMI changed significantly across the four time points (before treatment, 1st month after treatment, 2nd month after treatment, and 3rd month after treatment). Additionally, the interaction between group and time was significant ( $P\leq 0.0065$ ), indicating that the change in BMI over time was different for the three groups.

### Post-hoc analyses

For the main effect of group, the post-hoc Tukey HSD test elucidated the following pairwise comparisons. A: HFD versus HFD+GUTAC: A significant reduction in BMI was observed in the HFD+GUTAC group compared to the HFD group, with a mean difference of  $-0.0621$  ( $P=0.032$ ). The HFD versus SD+GUTAC groups: The analysis revealed a more pronounced effect, with a significant decrease in BMI in the SD+GUTAC group compared to the HFD group, underscored by a mean difference of  $-0.1012$  ( $P=0.045$ ). The HFD+GUTAC versus SD+GUTAC: When comparing the HFD+GUTAC group to the SD+GUTAC group, a significant mean difference of  $-0.0391$  was observed ( $P=0.0061$ ).

Analyzing the effect of time within each group, revealed that in the HFD group BMI increased significantly at each subsequent time point compared to the previous time point, indicating a steady increase in BMI over time (One-way repeated measures ANOVA:  $P=0.0003$ ). For the HFD+GUTAC group, there was no significant effect of time on BMI, suggesting that BMI did not change significantly over time in this group (One-way repeated measures ANOVA:  $P=0.489$ ). For the SD+GUTAC group, BMI decreased



significantly at each subsequent time point compared to before treatment (One-way repeated measures ANOVA:  $P=0.0002$ ). (Figure 1)

## Biochemical parameters

### Blood sugar levels

GUTAC significantly reduced blood sugar levels in obese rats. The HFD group had significantly higher blood sugar levels than both the HFD+GUTAC ( $P=0.0013$ ) and SD+GUTAC ( $P=0.0011$ ) groups. Blood sugar levels also increased significantly over time, with a significant difference observed at 90 days after treatment compared to before treatment ( $P=0.0003$ ).

### Renal function markers

BUN levels increased significantly over time, with the HFD group having significantly higher levels than the other groups at 45 and 90 days after treatment ( $P<0.05$ ). Creatinine levels were significantly higher in the HFD group compared to both the HFD+GUTAC

( $P=0.0002$ ) and SD+GUTAC ( $P=0.0009$ ) groups.

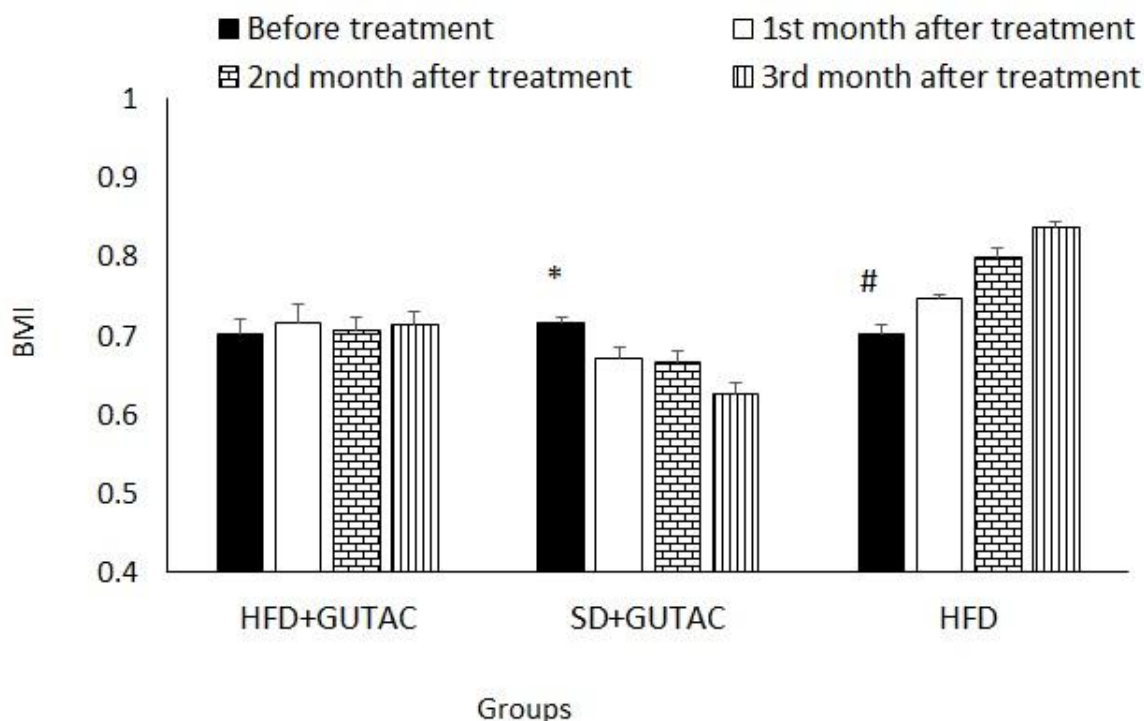
### Liver enzymes

While ALP levels did not differ significantly between groups, a significant increase was observed at 90 days after treatment compared to earlier time points ( $P=0.0002$ ). AST levels were significantly lower in the HFD+GUTAC group compared to the HFD group ( $P=0.0002$ ), and even lower in the SD+GUTAC group ( $P=0.0085$ ).

ALT levels were significantly higher in the HFD group compared to the HFD+GUTAC and SD+GUTAC groups at 45 and 90 days after treatment ( $P=0.01$ ).

### Lipid profile

Total cholesterol levels were significantly higher in the HFD group compared to both the HFD+GUTAC and SD+GUTAC groups at 45 and 90 days after treatment ( $P<0.001$  for both comparisons).



**Figure 1. Effect of GUTAC treatment on BMI**

\*: before treatment vs 1st, 2nd and 3rd month after treatment in SD+GUTAC group (all  $P<0.001$ ); #: before treatment vs 1st, 2nd and 3rd month after treatment (all  $P<0.001$ ).  $n=10$ rat/group

HDL levels decreased significantly over time in all groups, with a more pronounced decrease in the HFD group. LDL cholesterol increased significantly in the HFD group, reaching a 129% elevation at 90 days ( $P < 0.0001$ ). The HFD+GUTAC and SD+GUTAC groups showed only modest increases. Triglyceride levels increased significantly over time, with the HFD group exhibiting significantly higher levels compared to the HFD+GUTAC and SD+GUTAC groups ( $P < 0.001$  for both).

## Discussion

This preclinical study demonstrated the anti-obesity and metabolic benefits of a multi-herb preparation (GUTAC) containing Artemisia, licorice, fenugreek, nettle and green tea, in a rat model of high-fat diet-induced. The key findings were that GUTAC treatment, in combination with either a high-fat diet or standard diet, resulted in significantly lower body mass index, blood glucose, liver enzymes, total cholesterol, LDL cholesterol, and triglycerides compared to a high-fat diet alone. Additionally, GUTAC treatment prevented the decrease in HDL cholesterol associated with a high-fat diet and attenuated elevations in markers of kidney function like BUN and creatinine.

The reduced BMI with GUTAC treatment, especially when combined with a standard diet, indicates that the herbal preparation has anti-obesity effects and can prevent or reverse diet-induced weight gain. This is likely mediated through multiple mechanisms of action of the constituent herbs involving reduced appetite and calorie intake (fenugreek) (14,24), inhibited nutrient absorption (green tea) (25), improved glucose homeostasis and insulin sensitivity (fenugreek and nettle) (26), promotion of fat metabolism in adipose tissue (Artemisia) (27), and modulation of gut microbiota (licorice) (28).

The decreases in circulating lipids and liver enzymes highlight the hepato-protective effects of GUTAC against hepatic steatosis

caused by a high-fat diet. This is further supported by the histopathological analysis showing decreased fat deposition and damage in liver tissue. The herbs likely exert these effects through regulating key liver enzymes and signaling molecules involved in lipid metabolism (29-31).

By mitigating elevations in BUN and creatinine, the GUTAC combination prevented high-fat diet-induced kidney dysfunction. This renal protective effect may be attributed to the antioxidant and anti-inflammatory properties of ingredients like Artemisia (32) and green tea (33).

Despite the promising efficacy data, conflicting results have been reported in some studies on the constituent herbs which raise uncertainties regarding their therapeutic indices. High-dose licorice supplements were also linked with adverse events like hypertension in humans (34,35).

These discrepancies could arise from differences in bioactive constituent compositions between herbal preparations, dosing regimens, study model systems, sex and genetic or environmental modulators. The inconsistencies highlight the need for wider safety and efficacy profiling of multi-herb formulations across experimental models along with pharmacovigilance monitoring in human trials. Elucidating the specific active ingredients and mechanisms facilitating beneficial versus detrimental effects could help optimize the composition and dosage of preparations like GUTAC for clinical translation.

## Study limitations

Future studies need to evaluate different interactions (synergistic, additive and indifference) between GUTAC ingredients in obesity. There are other tests for evaluating effects of obesity on metabolism such as intraperitoneal glucose tolerance test (IPGTT) and oral glucose tolerance test (GTT). However, in this study we evaluated lipid

profile and blood sugar test to evaluate metabolic system.

## Conclusions

This preclinical study shows that the synergistic actions of multiple herbs targeting different mechanisms underlying obesity and metabolic syndrome are more effective than any single-agent approach. The results provide a strong scientific rationale for translating this promising multi-herb preparation into clinical trials for obesity, dyslipidemia, fatty liver disease, and diabetes. Further research should evaluate efficacy in humans and optimize the dosage and composition for maximum therapeutic benefits and safety.

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## Conflict of Interest

The authors declare no conflict of interest.

## Author contributions

MRSh designed the study. MM and AS measured and collected the data. MM, AS, MRM and JH analyzed the data and wrote the main parts of the manuscript. All the authors critically revised the manuscript, agree to be fully accountable for the integrity and accuracy of the study, and read and approved the final manuscript.

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