Phytochemical Study and Clinical Evaluation of Herbal Formula as Obesity Management

Wisam Ghazi Sanad[1], Saja Ismael Karkoosh[2], Ibrahim Salih Abbas[3], Qasim Allawi Bader[1]

[1]*College of pharmacy. Al-Zahraa University for Women. Email: wisam.sanad@alzahraa.edu.iq*
[2]*Department of pharmacy .AL-Mustafa University Colleg. Email: sajapharma83@gmail.com*
[3]*College of pharmacy. AL-Mustansiriya University. Email: dribrahim072@gmail.com*

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ABSTRACT

Objective: Obesity is a significant global public health issue, affecting 39% of overweight and 13% of obese adults, according to the World Health Organization (WHO). The objective of this study was included preliminary screening of main active compounds with clinical evaluation of herbal formula used in traditional medicine for obesity management. Methods. The methods for this study were included investigation of main active compounds in extracts of line seeds (Linum usitatissimum L.), Celery seeds (Apium graveoleus L.) Husk seeds (Plantago ovata L.) and marshmallow flower (Althea officinalis L.) Also this study contained the clinical evaluation of effects of herbal formula for obesity. The study involved two groups of participants who were given a herbal formula capsule for a duration of 8 weeks. The dosage consisted of one capsule (500mg) taken three times a day after meals. The second group was simultaneously treated with metformin, with a dosage of one tablet (500mg) provided twice daily after meals. Results: The results were referred to flavonoids glycosides which presented in all plants extracts, while other active compounds distributed differently among plants. The results of clinical study were referred to significant reduction in weight, BMI, Total cholesterol, Triglycerides, LDL – C and VLDL with significant increasing of HDL. Conclusion: The herbal mixture may exert beneficial effects on both body mass index and serum lipids profile in humans, which can be linked to its flavonoids and antioxidant components.

1. Introduction

Obesity is a common nutritional disorder its association with serious health disorders like coronary heart disease, hypertension, diabetes, pulmonary and dysfunction [1]. Obesity is a persistent metabolic condition that is influenced by various biological and environmental factors, as well as a sedentary lifestyle and a genetic susceptibility. The growing incidence of obesity and its detrimental health consequences pose significant global public health challenges [2]. Research has indicated that obese individuals with a higher body mass index (BMI) have a higher likelihood of developing coronary heart disease (CHD), cardiovascular disease (CVD), cancer, and premature death [3,4]. Medically, these patients are likely to exhibit elevated serum levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), along with decreased serum levels of high-density lipoprotein cholesterol (HDL-C). These characteristics have been established as risk factors for cardiovascular illnesses. The primary focus of treating high blood cholesterol is implementing a therapeutic lifestyle change (TLC) plan, which includes following a diet that is low in saturated fat and cholesterol, as well as engaging in weight reduction, maintaining regular physical exercise, and quitting smoking. All patients should undergo a thorough trial of TLC. However, high-risk patients who exhibit a low level of responsiveness to these adjustments should also receive concurrent pharmaceutical medication. An alternate method for reducing blood cholesterol levels is by the use of dietary adjuncts. Incorporating a variety of fiber-rich foods such as vegetables, legumes, whole grains, and fruits into your daily diet will effectively reduce blood cholesterol levels. Herbal medications contain a range of clinically useful components, which give them various pharmacological capabilities and perhaps less adverse effects compared to chemical drugs. [6] . Flax seeds are abundant in omega-3 fatty acids. Flax seeds include a group of minerals known as lignins, which possess potent antioxidant and estrogenic effects. These features may aid in the prevention of breast and prostate cancer, as well as other types of cancer [7]. Flax seeds exhibited favorable impacts on body composition. Whole-body flexion is an effective option for managing weight, especially for overweight and obese individuals seeking weight reduction [8]. Celery seeds (*Apium grave olens* L.) used for lowering obesity where showed the significant increase in the level of high-density lipoprotein cholesterol (HDL) and significant decrease in LDL was recorded [9] .Husk or (*plantago ovata*) seeds was gave significant effect on obesity and lipids profile

in animal study [10] .Marshmallow flowers extract were showed significant in reducing LDL-C with increasing HDL-C in animal study [11] .
The purpose of this study was to discover the primary active components and clinically evaluate the effectiveness of a mixture of the stated medicinal plants in preventing obesity.

2. Material and Methods :

Plant preparation:

The medicinal plant components utilized in this investigation were acquired from the local market in Baghdad, Iraq. The authentication of each plant was conducted by the national herbarium in the botany directorate of the Ministry of Agriculture.

Preliminary phytochemical Screening:

The plant extracts underwent preliminary phytochemical screening to qualitatively investigate the key types of secondary metabolites. One hundred grams of dried powdered plant material were individually defatted using 700ml of hexane. The defatted plant material was subjected to additional extraction using a soxhlet extractor with 80% ethanol (700ml). The ethanoic extracts were concentrated by evaporating them under reduced pressure using a rotary evaporator [12].

Test for Flavonoids

Ethanoic KOH 2% (2ml) was added to (1ML.) of ethanol extracts of each plant.

Test for Alkaloids

1mL. of aqueous were filtered and treated with 1mL. Dragendroff's reagent (Potassium Bismuth Iodide Solution).

Test for Tannins

Each plant extract, weighing -0.25g, was dissolved individually in 10mL of distilled water and subsequently filtered. The filtrates were treated with a 1% solution of ferric chloride (FeCl3) in water.

Test for Saponin

The ethanoic extracts of each plant, measuring 2 mL, were combined with 4 mL of distilled water in a test tube. The mixture was violently agitated in a graduated cylinder for a duration of 15 minutes.

.Test for Terpenes

An aliquot of hexane extract, was spotted on a thin layer chromatographic (TLC) plate percolated with silica gel. After development with chloroform: acetone (9:1) the TLC plate was dried and sprayed with vanillin sulphuric acid reagent which was prepared as follows: vanillin (1g) was dissolved in ethanol (100mL.) (solution 1) and concentrated H_2So4 (10mL.) was added drop wise to ethanol (90mL.) (solution 11). the plate was sprayed with solution 1, followed immediately by solution 11, then heating for 5-10 minutes at 110°C [13].

Coumarin Test

A small amount of ethanoic extract from each plant was applied to a filter paper, followed by the addition of one to three drops of a 1N NaOH solution. The filter paper is subsequently exposed to ultraviolet (UV) radiation.

Clinical Study Design:

This study was carried out under medical supervisor at Al-Razi center of medicinal plants. The medicinal plants used in this study were have approval from national center of herbal medicine / ministry of health / Iraq.

A total of one hundred obese patients were randomly allocated into two groups. The first group consisted of 60 obese patients, aged between 20 and 50 years. They were given a herbal formula capsule

for a duration of 8 weeks. The dosage was one capsule (500mg) taken three times day, after meals. The second group consists of 40 individuals who are obese, with ages ranging from 20 to 50. The patients were supplied metformin at a dose of one tablet (500mg) twice daily for a duration of 8 weeks, with one tablet taken in the morning and one in the evening.

Determination of Body mass Index (BMI)

The BMI describes relative weight in kilograms for height in meters : BMI=$\frac{WT(KG)}{HT(M2)}$ In general , the normal weight when BMI values between (18.5-24.9) , with underweight less than (18.5) , BMI overweight between (25-29.9) and obese BMI equal and more than (30) [14] .

Biochemical Parameters

Ten milliliters of venous blood samples were drawn by vein puncture from all participants after 12 hours of fasting all night as baseline sample before starting drug treatment and after 8 weeks as end line sample to follow up the difference in all research parameters. The biochemical parameters were included: Fast blood sugar (FBS) , The parameters of interest are total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride, and very low-density lipoprotein cholesterol (VLDL-C).

Statistical Analysis

All statistical analysis were achieved via the statistical package SPSS version 17.0 (SPSS,Inc.) . Least significant differences (LSD) for pair-wice comparison between two groups. The significant level for all tests was taken as p value less than (0.05) [15].

3. Results and Disscusion

Preliminary phytochemical screening

The results of phytochemical screening were summarized in table (1). These results revealed flavonoids, saponins and Terpenes in flax seeds, Celery seeds were contained flavonoids, tannins , terpenes and cumarines , husk seeds were contained flavonoids , alkaloids , tannins, terpenes , whil marshmallow flowers were contained flavonoids , alkaloids , tannins , saponins and terpenes .

Table (1) : Phytochemical profile of each plant extract used in herbal formula

Test	Flax seeds	Celery seeds	Husk seeds	Marshmallow flowers
Flavonoids	+	+	+	+
Alkaloids	–	–	+	+
Tannins	–	+	+	+
Saponins	+	–	–	+
Terpenes	+	+	+	+
Cumarins	–	+	–	–

Baseline characteristics of study patients

This study enrolled 100 individuals who were classified as obese, with a body mass index (BMI) equal to or greater than 30kg/m2. Prior to treatment, all patients were matched in terms of clinical parameters. A two-sample test was used to analyze a table (2) that showed no significant changes in these parameters between patients in the herbal formula group and the metformin (500mg) group.

Table (2) : Baseline characteristics of study patients

		Mean ±SE	P-Value
Age (Year)	Herbal formula N=60	34.15 ±	0.853
	Metformin = 40	33.4 ±	

Height (Meter)	Herbal formula N=60	2.57 ±	0.23
	Metformin = 40	2.55 ±	
Weight (Kg)	Herbal formula N=60	101.7 ±	0.291
	Metformin = 40	96.4 ±	
BMI (Kg/m^2)	Herbal formula N=60	40.73 ±	0.39
	Metformin = 40	39.45 ±	
N= Number of patients , SE= standard of error			

Table (3) shows that, after adjusting for covariates, there was a significant reduction (p value < 0.05) in weight and BMI in the herbal formula group compared to the metformin group, according to the covariance analysis.

Table (3) : Comparison of adjusted effect of treatment for weight and BMI of study groups according to covariance

	Treatment group	Adjusted Baseline	Adjusted End line Mean±SE	Outcome Mean±SE	P Value	% of difference
Weight (Kg)	Herbal Formula N=60	97.6340	93.123 ± 0.574	- 4.511 ± 0.574	0.012	4.6% ↓
	Metformin N=40	97.6340	95.585 ±0.709	- 2.049 ± 0.709		2.1% ↓
BMI (Kg/m2)	Herbal Formula N=60	38.127	36.450 ± 0.709	- 1.677 ± 0.709	0.011	4.4% ↓
	Metformin N=40	38.127	37.450 ± 0.224	- 0.677 ± 0.224		1.8% ↓
N= Number of patients , SE= standard of error						

In table (4) after adjustment of means of herbal formula group and metformin group according to the covariance analysis there is a non-significant difference P value > 0.05 between the two groups for fasting blood sugar , the lipid profile parameters including (total cholesterol , trigly cerieds , low density lipoprotein cholesterol , and very low density lipoprotein .

Table (4) : Comparis of adjusted means effect of treatment on metabolic parameters in study groups according to covariance

	Treatment group	Adjusted Baseline Mean±SE	Adjusted End line Mean±SE	Outcome Mean±SE	P Value	% of difference
FBS (mg/dI)	Herbal Formula N=60	95.98	93.98 + 1.43	- 2.00 + 1.420	0.883	2.08% ↓
	Metformin N=40	95.98	92.85 + 1.76	- 3.13 + 1.775		3.26% ↓
T.Cholesterol (mg/dI)	Herbal Formula N=60	175.88	165.7 + 3.65	- 10.18 + 3.65	0.910	5.79% ↓
	Metformin N=40	175.88	165.82 + 4.49	- 10.06 + 4.2		5.72% ↓
HDL (mg/dI)	Herbal Formula	38.91	41.2+ 1.13	2.20 + 1.10	0.540	5.90% ↓

	N=60					
	Metformin N=40	38.91	40.3 + 1.41	1.39 + 1.34		3.57% ↑
Triglyceride (mg/dI)	Herbal Formula N=60	136.5	123.410 + 5.73	- 13.09 + 5.59	0.793	9. 59% ↓
	Metformin N=40	136.5	126.1 +7.03	- 10.4 + 7.00		7.62% ↓
VLDL (mg/dI)	Herbal Formula N=60	26.4	23.8 + 1.52	- 2.6 + 1.09	0.793	9.85% ↓
	Metformin N=40	26.4	24.3 + 1.39	- 2.1 + 1.39		7.95% ↓
LDL (mg/dI)	Herbal Formula N=60	108.16	98.9 + 3.48	-9.26 + 3.51	0.820	8.56% ↓
	Metformin N=40	108.16	100.2 +4.29	- 7.96 + 4.29		7.36% ↓
N= Number of patients , SE= standard of error						

The data analysis in this study showed that the group of patients who received a combination of herbal formula, diet, and exercise experienced a significant reduction in weight and BMI compared to the group that received metformin along with diet and exercise. The analysis of covariance revealed a 4.6% reduction in weight and a 4.4% reduction in BMI for the herbal formula group. The group of patients who received metformin along with diet and exercise saw weight loss and a decrease in BMI, with a reduction percentage of 2.1% and 1.8% respectively. The activity of herbal formula may be belong to active compounds in medicinal plants were used in this study , such as flavonoids glycosides .Flavonoids glycosides in all medicinal plants Demonstrated anti-obesity and anti-diabetic benefits by the activation or introduction of several cytokines, enzymes, and metabolites to reduce inflammation, oxidative stress, and metabolic disturbances, therefore providing protection against obesity [16].A number of studies have demonstrated the potential health benefits of natural flavonoids in treating obesity and diabetic and show increased bioavailability and action on multiple molecular targets [17] .The beneficial or preventive effect of natural compounds against obesity is suggested in many studies , the potential of flavonoids in weight loss and obesity management could be through different influencing one or more of the pathways such as , lipid absorption , food intake and energy expenditure , pre-adipocyte differentiation and proliferation , lipogenesis and lipolysis and inflammatory response [18]. Flax seeds include lignins, which are potent antioxidants and a high supply of omega-3 fatty acids. They have the ability to reduce cholesterol levels, and this is likely owing to the fiber in flax seeds, which binds to bile salts and is subsequently eliminated by the body. Flax seeds have the ability to increase satiety and perhaps aid in weight management by regulating appetite.The recent investigations demonstrated that celery seeds led to a large increase in the level of high-density lipoprotein cholesterol (HDL-C) and a significant decrease in low-density lipoprotein cholesterol (LDL-C) levels in obese rats [20].In a separate study, it was found that adding P. oveta (Husk) to the diet can prevent endothelial dysfunction, hypertension, and obesity in obese Zucker rats. Additionally, it can improve dyslipidemia and maintain normal levels of adiponectin and TNF-alpha (Tumor necrosis factor alpha) in the rats' plasma [21].The application of mucilage led to a more significant reduction in weight compared to diet alone. Furthermore, the individuals who received mucilage therapy experienced a more significant decrease in plasma cholesterol and triglyceride levels.Also the mucilage of *A-officinalis* (marshmallow) flower extract used as a weight loss aid that blocks feelings of hunger [22] .

Conclusion

This herbal combination has a beneficial impact on BMI and the profile of lipids in the blood, which can be linked to its polyphenolic compounds, flavonoids, and antioxidant properties.
This formula also reduces the rate at which food leaves the stomach and enters the intestine for absorption by creating a thick gel-like substance, particularly with the inclusion of flax seeds, husk seeds, and marshmallow flower extract.

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