Antiobesity effect of Safoof Mohazzil, a polyherbal formulation, in cafeteria diet induced obesity in rats

Pooja Gupta, Jogender Mehla & Yogendra Kumar Gupta*
Department of Pharmacology, All India Institute of Medical Sciences, New Delhi 110 029, India

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Obesity is reaching epidemic proportions all over the world yet it lacks adequate treatment. Most of the drugs have failed either due to ineffectiveness or adverse effects. Complementary and alternative system of medicine is being used since ancient times. However, many of them have not been tested for efficacy and safety using modern scientific methods. Therefore, the antiobesity effect of Safoof Mohazzil, a polyherbal formulation, was evaluated in cafeteria diet induced obesity in female Sprague Dawley rats. Animals weighing 100–150 g were divided into four groups (n=8) i.e. standard pellet diet, cafeteria diet control, cafeteria diet + Safoof Mohazzil and standard pellet diet plus Safoof Mohazzil. The formulation was administered orally at a dose of 1 g/kg/day for 14 weeks. At the end of study, cafeteria diet significantly increased body weight, Lee’s index, lipid profile (cholesterol and triglycerides), insulin and leptin levels as compared to standard pellet diet control group. Fourteen week treatment with Safoof Mohazzil significantly prevented the increase in body weight, Lee’s index, lipid profile, insulin and leptin levels as compared to cafeteria diet control group without affecting food and water intake. Safoof Mohazzil had no adverse effect on hepatic transaminases, locomotor activity and motor coordination. The study provides evidence for antiobesity effect of Safoof Mohazzil.

Keywords: Cafeteria diet, Diet induced obesity, Herbal, Insulin, Leptin, Safoof Mohazzil, Unani medicine

*Correspondent author
Telephone: 91-11-26593282
Fax: 91-11-26588663
E-mail: yk.ykgupta@gmail.com; drgupta.pooja@gmail.com; jsmehlaaiims@gmail.com

Obesity is excessive accumulation of fat in the body associated with numerous complications such as cardiovascular disease, insulin resistance, type-2 diabetes mellitus, cancer and osteoarthritis1. The incidence and prevalence of obesity are rising both in developed and developing countries2. Pharmacological treatment of obesity has been a particularly challenging task. Drugs such as dexfenfluramine and sibutramine were banned in India in 1998 and 2011 respectively, due to their adverse cardiovascular effects and rimonabant in 2009 due to adverse neurological effects including suicidal behaviour. Orlistat is the only approved drug for long term treatment of obesity1. Ayurvedic and Unani medicines have been used for treatment of obesity for centuries. However, many of them have not been tested for efficacy and safety using modern scientific methods.

Safoof Mohazzil (SM), also spelled as Safuf Muhazzil, Sufoof-e-Mohazzil or Saffoof Muhazzil, is one such polyherbal formulation used by Unani physicians for treatment of obesity. However, no scientific data are available for this indication. Central Council for Research in Unani Medicine (CCRUM), Department of AYUSH, Ministry of Health and Family Welfare, Govt. of India sponsored the present study to develop a scientific body of evidence. The formulation for the study was supplied by the Directorate General of Unani Council and was informed to contain nankhawah (Ptychotis ajowan) seed, marzan josh (Origanum majorana) stem, tukhme badiyan (Foeniculum vulgare Mill) seed, zeera siyah (Carum carvi) seed, lakh maghsool (Coccus lacca), bura armani (Armenian bole) and Berg sudab (Ruta graveolens Linn.) leaf.

Ptychotis ajowan also known as ajwain (Hindi), has stimulant, carminative and antispasmodic actions. It is used in Unani system for digestive problems like flatulence and for stimulating digestion. Origanum majorana extract has been used in folklore for the treatment of hay fever, sinus congestion, indigestion, asthma, stomach pain, headache, dizziness, colds, cough, and nervous disorders3-6. Foeniculum vulgare commonly known as fennel possess carminative, hepatoprotective, antioxidant, emmenagogue and galactagogue properties7-9. In these traditional
therapies, zeera siyah (known as Carum carvi) seeds are prominently considered carminative, eupetic, antispasmodic, astringent and used in the treatment of mild digestive disorders, diarrhea, dyspepsia, flatulence, morning sickness, colic, dyspeptic headache and bloating and to improve liver function. Aqueous extract of Carum carvi showed potent lipid lowering activity (hypotriglyceridemic and hypocholesterolemic) in both normal and STZ-diabetic rats after single and repeated oral administration. Coccus lacca stimulates digestive system, has anti-inflammatory properties and is used in the Unani system for treatment of obesity, blood, liver and kidney disorders. Armenian bole commonly known as Bura Armani, is used for food poisoning, heartburn, indigestion and nausea. Ruta graveolens leaf is used as a carminative in the Unani system. Methanolic extract of Ruta graveolens has been shown to possess antioxidant, anti-inflammatory and hypocholesterolemic action.

This study has been carried out to evaluate the antiobesity activity of Safoof Mohazzil in diet induced obesity in rats. Daily food and water intake, gain in body weight, blood glucose level, serum lipid profile, leptin and insulin levels have been evaluated to study the obesity development in rats.

Material and Methods

Animals—Female Sprague Dawley rats weighing 100–150 g were obtained from the Central Animal Facility of the Institute. The rats were housed individually under normal laboratory conditions with natural light-dark cycle, humidity and controlled temperature (20–25 °C). The animals were acclimatized to the environment for a week prior to experimentation with free access to water and pellet diet for rats (week 0). The study was approved by Institutional Animal Ethics Committee (Letter No. 463/08 dated 22.02.09). The study was conducted in accordance with the Indian National Science Academy Guidelines for care and use of animals in scientific research.

Preparation of formulation—Each 10 g of the formulation contained nankhawah (Ptychotis ajowan) seed 2 g, marzan josh (Origanum majorana) stem 0.5 g, tukhme badiyan (Foeniculum vulgare Mill) seed 2 g, zeera siyah (Carum carvi) seed 2 g, lakh maghsool (Coccus lacca) 1 g, bura armani (Armenian bole) 0.5 g and Berg sudab (Ruta graveolens Linn.) leaf 2 g. The ingredients were individually dried in shade, powdered and then mixed in the mentioned proportion. The finished formulation was a fine powder, greenish brown in colour.

Induction of obesity and drug treatment—The animals were randomly divided into four groups of 8 animals each (Day 1, Week 1). Group I was fed standard pellet diet (normal control). Group II was fed cafeteria diet (obesity control). Group III was fed cafeteria diet along with oral SM 1 g/kg/day (study group). Standard diet along with SM 1 g/kg/day was given to group IV (drug per se group). A mixture of chocolates, butter cookies and dry coconut was used as cafeteria diet and supplemented with standard pellet diet to provide the micronutrients. Food and water was given ad libitum. The dose needed for rats was calculated on the basis of its human dose informed by Unani physicians using the formula: animal dose (g/kg) = (total human dose (10 g/day) / average body wt (60 kg)) * Conversion factor (6.2). Safoof Mohazzil was suspended in 1% carboxymethyl cellulose solution as it is insoluble in water. It was administered orally by gavage at 1 g/kg, in volume not greater than 1.0 mL/100 g body weight daily for 14 weeks (Day 1, Week 1 till Day 98, Week 14). The animals were observed for daily food and water intake and weekly change in body weight. Lee’s index, behavioural parameters (locomotor activity and motor coordination), lipid profile (total cholesterol (TC), high density lipoprotein (HDL-C), triglycerides (TG)) and blood levels of glucose, leptin, insulin and hepatic transaminases (serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT)) were measured at the end of week 0 and week 14. Care was taken to carry out all experiments between 09.00–11.00 h to minimize the chronobiology effects.

Measurement of change in body weight, food and water intake and Lee’s index

Change in body weight—Body weight of animals in each group was recorded on day 1 (before starting drug treatment) and on alternate days for 14 weeks before giving the food and water.

\[ \Delta BW\% = \left( \frac{BW \text{ at the end of ‘n’ week (g)} - BW \text{ on day 1 (g)}}{BW \text{ on day 1 (g)}} \right) \times 100 \]

Food consumption—All animals were housed individually in polypropylene cages. Measured amount of food was kept in each cage daily. Next day the remaining food was weighed. For cafeteria diet, each item was provided in excess along with an excess amount of standard pellet diet. Food
Consumption per 100 g of body weight of animals was calculated as:

Diet consumed (g) = total diet provided (g) – total diet remaining (g)

Diet consumed per 100 g body weight (g) = (Diet consumed in ‘n’ week / mean body weight in that week) × 100

Water intake—Rats were provided with measured quantity of water each morning. Left over volume was noted next morning to calculate water intake per 100 g of body weight.

Water consumed (mL) = Water provided (mL) – Water remaining (mL)

Water intake per 100 g of body weight (mL) = (Water consumed in ‘n’ week / mean body weight in that week) × 100

Lee’s Index—Lee’s index in animals is considered analogous to body mass index in humans. It has been shown to correlate well with the body fat content of the animals. It is calculated as

LI = [(BW (g))^{1/3} / Nasoanal length (mm)]

Lee’s index was calculated at the end of week 0 (baseline) and week 14 (after administration of last dose of SM). The Naso-anal length of the rats was measured under anesthesia on the day of blood collection.

Behavioral parameters:

Closed field test—The locomotor activity was assessed on week 0 (baseline) and week 14 (after last dose of SM) using digital photoactometer. The number of cutoffs of the infrared light beams was counted for each rat for 5 min and taken as the measure of locomotor activity.

Rota rod test—Motor coordination in rats was assessed using rota rod on week 0 (baseline) and week 14 of administration of SM. Each rat received a training session on the rota rod at a constant speed of 8 rpm until they achieved the criteria of remaining on the rotating spindle for 60 s. After training, the time to fall from an accelerating rod was recorded.

Biochemical parameters:

Fasting blood glucose level—The animals were fasted overnight for estimating blood glucose level at the end of week 0 and week 14. Its estimation was carried out using commercially available glucometer (Onetouch Ultra) which estimates glucose based on the oxidase method. Glucometer was used in this study since it uses only a small drop of blood, gives instant results and the glucose levels were found to be within ± 10% of those estimated by the institute laboratory procedure.

Estimation of hepatic transaminases, lipid profile, insulin and leptin—At the end of week 0, blood was collected from the orbital plexus using capillary method under light ether anesthesia for estimation of SGPT, SGOT, lipid profile, insulin and leptin. At the end of week 14, the animals were euthanized with ether and blood was collected by direct cardiac puncture. Serum was separated and stored at –80°C until analyzed. Serum TC, HDL-C and TG were measured using commercial kits (Vital Diagnostics (P) Ltd, India) using semi-autoanalyzer. Serum leptin and insulin levels triglyceride (TG) were measured using ELISA kits (Mercodia AB, Sweden for insulin and Medinagnost GmbH, Germany for leptin).

Statistical analysis—Results are expressed as means±SD. One way analysis of variance (ANOVA) with Bonferroni post hoc test was carried out using SPSS statistical software package version 13.0. A P<0.05 was considered as significant.

Results

Effect of Safoof Mohazzil on body weight, food and water intake—There was a significant difference in percentage change in body weight among the groups [F (3,27) = 53.422, P = 0.0001]. On post hoc analysis, there was a significant increase in % change in body weight in group II as compared to group I, 3 weeks onwards (Table 1). The cafeteria diet significantly increased % change in body weight from 62.09 ± 14.43 in group I to 139.94 ± 21.45 in group II (P<0.001) at 14th week of study in comparison to baseline. Treatment with SM significantly prevented the increase in % ∆BW in group III as compared to group II from 7th week where as no significant difference was found between group I and IV (Table 1). Treatment for 14 weeks significantly prevented the increase in % ∆BW from 139.94 ± 21.45 in group II to 72.63 ± 6.95 in group III (P<0.001). Safoof Mohazzil did not affect BW in group IV as compared to group I (P>0.05) (Table 1).

Food and water intake by animals was measured every day. Average food and water intake per week are given in Table 1. After statistical analysis, it was found that difference in food and water intake among the groups throughout the study was not significant (P>0.05). Safoof Mohazzil also did not cause any significant change in total food or water intake in group III and IV as compared to group I and II (P>0.05).
<table>
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<tr>
<th>No. of weeks</th>
<th>Food intake (g)/ 100 g body weight</th>
<th>Water intake (mL)/ 100 g body weight</th>
<th>Change in body weight (%)</th>
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<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
</tr>
<tr>
<td>1</td>
<td>11.92±1.06</td>
<td>9.42±2.03</td>
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<td>2</td>
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<tr>
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<td>14</td>
<td>8.70±0.56</td>
<td>7.25±1.64</td>
<td>7.28±1.22</td>
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</table>

Group I= Standard pellet diet control, Group II= Cafeteria diet control, Group III= Cafeteria Diet plus 1g/kg/day Safoof Mohazzil, Group IV= Standard pellet diet plus 1g/kg/day Safoof Mohazzil

P values: *<0.05; !<0.01; #<0.001; * and - as compared to group I; ! and ^ - as compared to group II
Effect of Safoof Mohazzil on the Lee’s index—At the beginning of experiments, Lee’s index of the treatment groups did not differ significantly \[F (3, 27) = 82.081, P = 0.279\] as analyzed by one way ANOVA. After the end of study, post hoc statistical test showed significant \((P<0.001)\) increase in LI from 0.029 ± 0.00022 in group I to 0.033 ± 0.00025 in group II (Fig. 1). Co-administration of SM significantly decreased the LI from 0.033 ± 0.00025 in group II to 0.029 ± 0.00023 in group III \((P<0.001)\).

Safoof Mohazzil treatment did not cause any significant change in the LI group IV in comparison to group I (Fig. 1).

Effect of Safoof Mohazzil on behavioural parameters—The locomotor activity of animals was tested by using digital actophotometer. After one way ANOVA, it was found that the difference in the locomotor activity among the groups was non-significant \[F (3, 27) = 0.433, P = 0.731\] before the drug treatment. The locomotor activity was also tested after the last dose of SM. The results indicate that there was no significant change in the locomotor activity among the groups \[F (3, 27) = 0.564, P = 0.643\] (Table 2).

Motor incoordination in animals was tested using rotarod apparatus. One way ANOVA revealed that there was no significant difference in time of falling of animals among the groups \[F (3, 27) = 0.220, P = 0.882\] at baseline. After the last dose of SM, motor incoordination in animals was again tested. No significant difference was found in the time of falling of animals from the spindle of rotarod among the group III as compared to group I \[F(3,27)=1.087, P=0.372\] (Table 2).

Effect of Safoof Mohazzil on fasting glucose, SGOT, SGPT, TC, HDL-C and TG levels—No significant difference was found in the basal levels of fasting glucose, SGOT, SGPT among the groups \((P>0.05)\). Cafeteria diet did not cause any significant change in the fasting glucose, SGPT and SGOT levels as compared to group I \((P>0.05)\). At the end of week 14, there was no significant difference \((P>0.05)\) in fasting glucose, SGPT and SGOT levels in groups III and IV in comparison to groups I and II (Table 2).

![Fig. 1—Comparison of Lee’s index of rats before and after 14 weeks of study. The drug treatment given was 1 g/kg/day Safoof Mohazzil. Data are presented as mean ± SD. ***P<0.001, a-as compared to standard pellet diet control; b-as compared to cafeteria diet control](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
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<th>Group III</th>
<th>Group IV</th>
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<tr>
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<td>138.7 ± 26.29</td>
<td>137.1 ± 34.29</td>
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<td>Rota rod</td>
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<td>146.4 ± 20.77</td>
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<td>Fasting blood glucose (mg/dl)</td>
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<td>100.6 ± 4.03</td>
<td>102.4 ± 9.243</td>
<td>100.5 ± 8.67</td>
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<tr>
<td>Serum SGOT (IU/L)</td>
<td>105.6 ± 17.65</td>
<td>107.9 ± 14.29</td>
<td>105.4 ± 13.99</td>
<td>111.1 ± 15.86</td>
</tr>
<tr>
<td>Serum SGPT (IU/L)</td>
<td>64.2 ± 18.54</td>
<td>66.5 ± 12.38</td>
<td>63.6 ± 17.18</td>
<td>64.1 ± 7.66</td>
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<tr>
<td>Serum TC (mg/dl)</td>
<td>74.1 ± 1.49</td>
<td>72.1 ± 2.82</td>
<td>72.2 ± 1.99</td>
<td>81.7 ± 1.93**</td>
</tr>
<tr>
<td>Serum TG (mg/dl)</td>
<td>83.8 ± 4.86</td>
<td>82.04 ± 1.40</td>
<td>81.2 ± 2.56</td>
<td>101.7 ± 2.93**</td>
</tr>
<tr>
<td>Serum HDL-C (mg/dl)</td>
<td>36.04 ± 1.63</td>
<td>35.6 ± 0.72</td>
<td>36.3 ± 1.53</td>
<td>30.2 ± 0.92**</td>
</tr>
</tbody>
</table>

Group I=Standard pellet diet control, Group II= Cafeteria diet control, Group III=Cafeteria Diet plus 1g/kg/day Safoof Mohazzil, Group IV=Standard pellet diet plus 1g/kg/day Safoof Mohazzil

* A significant increase at \(P<0.001\)

* When compared to standard pellet diet control

* When compared to cafeteria diet control
The basal levels of TC, TG and HDL-C did not differ significantly \( (P>0.05) \) among the groups. A significant rise in TC and TG levels in group II as compared to group I was observed at the end of 14 weeks \( (P<0.001) \). The formulation when used for 14 weeks, significantly decreased \( (P<0.001) \) the TC and TG levels in group III as compared to group II (Table 2). No significant difference was found in between group IV and group I \( (P>0.05) \).

There was a significantly decline in the HDL-C level in group II as compared to group I \( (P<0.001) \). Treatment with SM significantly increased HDL-C level in group III as compared to group II \( (P<0.001) \). Safoof Mohazzil did not cause any significant \( (P>0.05) \) change in the HDL-C level in group IV as compared to group I (Table 2).

**Effect of Safoof Mohazzil on insulin level**—The basal insulin levels did not differ significantly amongst the groups as indicated by one way ANOVA. At the end of the study period, a significant difference was found in between the treatment groups at the end of the study \( [F(3, 14)=35.715, P=0.0001] \). Bonferroni post hoc analysis revealed significant rise in insulin level in group II in comparison to group I \( (P<0.001) \). Leptin level increased from 464.90 ± 76.74 pg/mL in group I to 2346.50 ± 347.25 pg/mL in group II (Fig. 3). Treatment for 14 days significantly \( (P<0.001) \) decreased the leptin level from 2346.50 ± 347.25 pg/mL in group II to 477.25 ± 160.82 pg/mL in group III (Fig. 3). Safoof Mohazzil did not cause any significant change in leptin levels in group IV in comparison to group I (Fig. 3).

**Discussion**

Obesity affects nearly one third of the adult population in developed countries. An excess accumulation of fat in the body leads to obesity which in turn is a risk factor for a multitude of other disease conditions like hypertension, myocardial infarction, peripheral vascular disease and diabetes. Herbal medicines are being looked up for treatment of obesity due to a long standing experience with them in the traditional systems of medicine and failure of many conventional medicines. There are various preclinical and clinical studies in which efficacy of herbal drugs have been reported\(^{14-17}\). Safoof Mohazzil is one such Unani formulation which is used in India since the ancient times. This study evaluated the anti-obesity effect of this formulation in experimental model.

Cafeteria diet induced obesity model was selected for the study since it is a well established animal model for obesity which simulates the imbalance between energy intake and expenditure in humans\(^{18}\). Chocolate, coconut and butter cookies were selected as part of the cafeteria diet since these are commonly...
consumed food items in the modern lifestyle and represent a diet rich in sugar, fat and carbohydrates. Young female rats (low weight range) were selected as they have been shown to be more prone to weight gain. Increase in body weight in cafeteria diet control group started from 2 weeks of the study in comparison to standard pellet diet control. The significant increase in body weight, Lee’s index, lipid profile, serum leptin and insulin levels demonstrate successful development of obesity in the study.

A significant increase in body weight in cafeteria diet group is in accordance with results of previous studies. High fat diet increases the expression of fatty acid catabolism related genes in the small intestine which is associated with development of obesity. Safoof Mohazzil treatment prevented the increase in body weight. However the effect was significant from week 7 onwards. Lee’s index, also known as obesity index, has been shown to correlate well with % body fat especially in the diet induced obesity models. In the present study, Lee’s index was significantly increased in obese animals which was prevented by treatment with SM. However, SM per se did not alter the Lee’s index suggesting a probably lack of effect in normal animals.

Fluctuations were observed in food and water intake over 14 week treatment period. At the end of study, the food and water intake did not significantly differ between groups suggesting that Safoof Mohazzil neither causes anorexia nor diuresis. Its anti-obesity effect might be thought to be due to improved digestion, energy metabolism or lipolysis as suggested for other antiobesity herbs. Pancreatic lipase is well known for its role in fat metabolism and absorption of lipolysis products. Although, lipase inhibitory effect of Unani formulation was not evaluated but its involvement in antiobesity effect of this formulation cannot be ruled out. The studied Unani formulation might decrease fat absorption by inhibiting its digestion.

Leptin level has been reported to be increased in obese subjects and its plasma level fluctuates with fasting and changes in insulin levels. Previous studies have reported that high fat diet induced obesity and insulin resistance leads to increase in plasma leptin levels. Adipose tissue produces leptin and its level is associated with the weight of adipose tissue. Have suggested that high fat diet for a long time may result in increased serum leptin. In the present study also, a significant increase in insulin and leptin levels was observed in cafeteria diet group. Safoof Mohazzil treatment prevented the cafeteria diet induced adverse changes in insulin and leptin levels suggesting its potential usefulness in metabolic syndrome. Studies using genetic models may further enhance understanding of the mechanism of action of Safoof Mohazzil.

High fat diet induced hyperlipidemia, hyperinsulinemia and increased levels of leptin are well reported. High fat diet increases the expression of fatty acid catabolism related genes in the small intestine which is associated with development of obesity. In the present study also, cafeteria diet increased the TC and TG levels and decreased the HDL-C levels. Safoof Mohazzil significantly decreased the TC and TG levels and increased the HDL-C levels as compared to cafeteria diet group. Fasting blood glucose and serum SGOT and SGPT levels were not altered in cafeteria diet control group as compared to standard pellet diet control. Safoof Mohazzil treatment also did not cause any change in fasting glucose, SGOT and SGPT levels in any groups.

Motor coordination and locomotor activity of animals were evaluated in the present study using rota rod and photoactometer, respectively. The results indicate that high fat diet does not alter motor activity. Safoof Mohazzil per se also does not adversely affect motor activity. The study demonstrates the potential antiobesity effect of Safoof Mohazzil. However the study has few limitations. The study has a standard diet control; cafeteria diet control and drug per se control groups. However, no positive control group was included in the study as the exact mechanism of action of the test formulation is not known. The study does not demonstrate the dose response relationship for the formulation. Safoof Mohazzil is being used by the Unani physicians at a single dose of 5–7 g twice daily. Hence, for this study, only a single dose equivalent to the human dose was used. The formulation used in the present study is being prescribed by Unani physicians for weight loss since ages. It is based on the traditional Unani system of medicine. As reported by Unani physicians, the preparation is safe for human use. However, Safoof Mohazzil has not undergone formal toxicity studies. It was not standardized due to presence of many ingredients each of which contains multiple active constituents.

The results of the present study indicate that Safoof Mohazzil, a polyherbal Unani formulation, prevents...
the increase in body weight without affecting food and water intake; alters the lipid profile favorably and prevents rise in insulin and leptin levels. These actions suggest a potential antiobesity effect of Safoof Mohazzil which needs confirmation using other obesity model and it may be further developed for management of obesity.

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None of the authors have competing financial interests either actual or potential.

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