Potential Effect of Fisetin in A sample of Obese Diabetic Patients in Iraq

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ABSTRACT

Background: Fisetin has been shown previously to attenuate obesity and regulate glucose metabolism *in vitro* and *in vivo*. Objective: To assess the potential effect of the dietary supplement (Fisetin) on obese patients with type 2 diabetes. Methods: This randomized, single-blind, controlled study was carried out in Iraq and investigated the effects of 8 weeks of fisetin (100 mg/day) on body weight, BMI, WC, WHR, WHtR, HOMA-IR, and serum levels of fasting glucose, insulin, leptin, adiponectin and creatinine. In this study, 51 Iraqi obese diabetic patients (21 males and 30 females) were randomized into either the fisetin or control group. The fisetin group received fisetin 100 mg/day capsules in combination with their previous treatment, that is metformin (500 mg t.i.d) tablets, while, the control group already received metformin (500 mg t.i.d) tablets in an 8-week trial. Results: there was significant decrease in body weight, BMI, WC, HOMA-IR, serum levels of FBG, insulin and leptin and a significant increase in serum adiponectin level in the fisetin group at the end of the study in comparison with the baseline values. Regarding WHR, WHtR, and serum creatinine they were diminished insignificantly by fisetin supplementation compared with baseline values. However, a significant difference in body weight, BMI, WC, serum levels of FBG, HOMA-IR, leptin and adiponectin was observed in the fisetin group when compared to the control group. The changes in WHR, WHtR, and serum levels of insulin and creatinine were not statistically significant compared to the control group. According to these results, fisetin could aid in the treatment of obesity and improve the diabetic status of obese diabetic patients, suggesting it as a novel complementary anti-obesity agent for these patients and warranting further studies.

Keywords: fisetin, obesity, type2 diabetes, leptin, adiponectin

1. Introduction

Obesity is a long-term metabolic condition that affects both adults and children. One of the top causes of mortality is obesity, which is linked to a variety of non-communicable illnesses, including type 2 diabetes. Excessive adiposity characterizes the majority of T2D patients, particularly in the visceral region, where adipocytes with a high lipolytic rate create an excess of FFAs that are largely insulin resistant (1,2). In 2015, the adult obesity rate in Iraq was 33.50% (25.60 % for men and 42.60 % for women) (3). Type 2 diabetes mellitus has also risen in parallel with the obesity pandemic, and is now regarded as a significant obesity co-morbidity. According to current epidemiological studies, obese people account for 85% of T2DM patients, and more than 300 million people worldwide will acquire T2DM as a result of obesity by 2025 (4).

In light of the high incidence of T2D and obesity, new treatment methods targeting pathomechanisms common to both illnesses are urgently required. Clinical trials have been conducted on a variety of anti-obesity medications. However, such medications can have unanticipated adverse effects in addition to their anti-obesity efficacy. Because of these adverse effects, there is a significant desire for therapeutically effective but safe anti-obesity medications. Thus, the use of some medical plants and their derivatives has a likely mode of action as natural anti-obesity agents with no or minimum adverse effects. Plant-derived natural chemicals have a wide range of biological and pharmacological properties, including anti-inflammatory and antioxidant properties. Furthermore, when such chemicals are utilized as dietary supplements, their actions are frequently beneficial for weight management and other related problems (5,6).

Fisetin is a bioactive flavonol molecule found in a variety of fruits and vegetables, including strawberries, apples, dates, mangoes, kiwi, grapes, tomatoes, onions, cucumbers, nuts, wine, and

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various Fabaceae and Anacardiaceae family trees and shrubs, such as the Japanese fruit wax tree (Rhus succedanea). Fisetin has become so popular as a nutraceutical or dietary supplement in the Western world that it now possesses various bioactive qualities and is regarded as a health-promoting element (7). Even at large doses, there is no evidence of fisetin causing side effects (8). It has been claimed that fisetin medication can lessen the etiology of dietary obesity through a variety of ways. Fisetin supplementation has been shown in studies to reduce the risk of obesity by suppressing adipocyte differentiation and proliferation. When fisetin was given to 3T3-L1 undifferentiated fibroblast cells, differentiation in adipocytes was decreased (9). Fisetin therapy has been shown to reduce adipocyte differentiation and proliferation by reducing mitotic clonal expansion. The expression of certain essential cell cycle promoters, including as cyclin A, cyclin D1, and cdk4, has been suppressed by fisetin therapy. Furthermore, by continually supporting the G0 phase, fisetin has increased the cell cycle inhibitor p27 expression (10). Fisetin also is believed to have a role in diabetes management as a natural alternative to current diabetic medications with fewer side effects. In diabetic animal models, fisetin has been shown to reduce plasma glucose levels via potential glycolysis, inhibition of gluconeogenesis, and increased storage of glycogen and may function as a powerful GLUT2 intestinal sugar transport inhibitor (11). In another study, oral fisetin treatment of rats resulted in metabolic alterations that improved diabetic tolerance via altering glucose metabolism enzymes (12). Many of fisetin's actions have the ability to decrease metabolic problems linked with diabetes mellitus, thus it's conceivable that it may help to alleviate or prevent obesity (11). However, despite the fact that obesity plays an important role in the pathogenesis of diabetes and that fisetin has been shown to have anti-obesity and anti-diabetic effects in many laboratory and animal studies, there has been no clinical trial to evaluate the potential impact of fisetin as a natural food substance on obesity and diabetes. Accordingly, this study aims to evaluate if fisetin supplementation may have an effect on obesity and related factors in obese type 2 diabetic individuals.

2. Patients and Methods

This study was a randomized, single-blind, controlled study to assess the effects of 8 weeks of fisetin (100 mg/d) supplementation in obese people with type 2 diabetes. The study was conducted between October 2020 and December 2021 in Baghdad, Iraq and was approved by the local ethical committee of the College of Pharmacy/AL-Mustansiriyah University and the Ministry of Health. A total of 60 obese diabetic patients were enrolled in the study during their visit to the National Diabetes Center for Treatment and Research/Al-Mustansiriyah University. Only 51 patients (30 women and 21 men) completed the study. All participants received detailed clarification regarding the intent of the study and provided their informed consent.

Major inclusion criteria were BMI of ≥30 kg/m2 and a waist circumference of more than 88 cm for women and more than 102 cm for men, and had type2 diabetes mellitus, age 18-50 years. Important exclusion criteria were women who were pregnant, lactating or on contraceptives, as were patients with severe health problems such as renal disease, liver disease, cardiovascular disease, uncontrolled thyroid function, women with polycystic ovary syndrome, Cushing syndrome and diabetic complications or other chronic health conditions; patients who were currently taking hypoglycemic agents other than metformin; patients who were already taking fisetin supplements or other herbal supplements; and patients who had previously taken anti-obesity drugs before the time of enrollment; a history of allergic reactions to fisetin or strawberries; inability to provide informed consent, i.e., unwillingness to participate in the study procedure.

3. Randomization and Intervention

The study involved an 8-week treatment. The patients were randomized into fisetin or the control group. For the fisetin group, 26 patients take 100 mg of Wax Tree derived fisetin supplement once daily, which was added to their previous treatment (500 mg of metformin three times daily). For the control group, 25 patients already take 500 mg of metformin three times daily. Based on the FDA model of converting an in vivo dose to a safe human equivalent, a safe dose of 100 mg of fisetin per day was adopted (13). In this study, randomization with assignment was obscured by opaque prenumbered envelopes. At the end of the eight weeks, compliance was measured by counting the returned capsules. The patients were phoned once a week to confirm that they were taken the capsules as directed.

4. Measurements

Anthropometric Measurements

Patients wore light indoor attire and their body weight was measured using a weighing scale with a 0.5 kg accuracy. Using a stadiometer to the closest 0.5 cm, standing height was measured without shoes on a flat surface with weight distributed evenly on both feet and heels together. To measure height, a vertical board with an attached metric rule and a horizontal headboard that could come into contact with the highest point on the head were utilized. The BMI was computed by dividing one's weight in kilograms by the square of one's height in meters (kg/m2). To the nearest 0.5 cm, the waist circumference was measured halfway between the lower edge of the last perceptible rib and the top of the iliac crest. The patient wore light clothing and

stood with her feet close together, arms at her sides, and her body weight equally distributed. By dividing the waist circumference by the hip circumference, the waist-hip ratio (WHR) was computed. By dividing the waist circumference by the measured height in centimeters, the waist-height ratio (WHtR) was determined.

Other Blood Test and Biochemical Data Measurement

Before and after the 8-week treatment, all blood tests and other biochemical data were collected and tested in the standard laboratory at the National Diabetes Center for Treatment and Research at Al-Mustansiriyah University. After a 12-hour fast, blood samples were collected in the morning. The glucose oxidase technique was used to determine blood glucose levels. Depending on the type of Jaffe reaction, serum creatinine was measured using a commercial kit (Human, Germany). Sandwich-Enzyme-Linked Immunosorbent Assay (ELISA) kits were used to assess serum insulin (Monobind Inc, USA) and leptin (Demeditec, Germany), as well as adiponectin (mybiosource, USA). The HOMA method has been used to compare variations in insulin resistance profiles (HOMA-IR). HOMA-IR = (Fasting serum insulin (μIU/ml))* (Fasting blood glucose (mg/dl))/405.

5. Statistical Analysis

The data was analyzed using the accessible statistical program SPSS-27 (Statistical Packages for Social Sciences-version 27). The data was given in the form of a mean and standard deviation. The significance of the difference between various means (quantitative data) was assessed using the Paired-t test for the difference between paired observations or two dependent means and the Students-t test for the difference between two independent means. The chi-squared test was used to compare categorical variables (numbers of patients). Statistical significance was evaluated when the P value was equal to or less than 0.05.

6. Results

A total of sixty patients participated in this study. Five patients in the control group withdrew from the study for unknown reasons, and four patients from the fisetin group were excluded from the study. Among the four patients, one failed to complete the study due to a lack of effectiveness, while the other three failed due to a lack of compliance. Thus, 51 participants were considered for the final analysis. The mean and SD of age for all the participants were 35.87±8.7 and 38.62±7.3 years, with the male-to-female percentage being (38.4%: 61.5 %, and 44.0%: 56.0%) for the fisetin and control groups, respectively. The demographics and characteristics of the study participants in the fisetin and control groups are shown in (table 1). At baseline, no significant differences existed in the demographics, characteristics of participants, or variable values between the two groups. The changes in anthropometric measurements, HOMA-IR, and the serum levels of FBG, insulin, leptin, adiponectin and creatinine (at baseline and after 8 weeks) and between the fisetin and control groups, are summarized in (table 2).

The within-groups (at baseline and after 8 weeks) analysis showed that there was significant decrease in body weight from 89.93±12.49 kg to 85.41±11.19 kg , BMI from 34.35±4.73 kg/m2 to 32.62±3.46 kg/m2 , WC from 103.71±11.25 cm to 100.37±10.55 cm, HOMA-IR from 8.28±3.61 to 5.45±2.05, serum levels of FBG from 158.58±45.31 mg/dl to 130.16±24.02 mg/dl, insulin from 21.17±7.98 μIU/ml to 16.97±5.12 μIU/ml and leptin from 14.67±3.54 ng/ml to 11.99±3.03 ng/ml and a significant increase in serum adiponectin level from 20.40±9.20 ng/ml to 32.61±14.13 ng/ml in the fisetin group at the end of the study in comparison with the baseline. Regarding WHR, WHtR, and serum creatinine they were diminished insignificantly by fisetin supplementation compared with baseline values from 0.90±0.08 to 0.89±0.07, from 0.64±0.07 to 0.62±0.06, and from 0.77±0.12 to 0.70±0.15, respectively. However, there were significant changes during the study in the serum levels of glucose in the control group. Additionally, there were no significant changes in the anthropometric parameters and other assessed biochemical factors in the control group. Between-groups (fisetin and control) comparison of changes in the anthropometric parameters, biochemical factors, and adipokines in the fisetin and control groups showed a significant difference in body weight, BMI, WC, serum levels of FBG, HOMA-IR, leptin, and adiponectin, but no significant changes in WHR, WHtR, or serum levels of insulin and creatinine (table2).

Table 1: Baseline demographic and characteristics of the patients (n = 51)

Study variables	Fisetin control p-value
Age (years)	35.87±8.7 38.62±7.3 NS
N	26 25 NS
Gender N (%)	
Male	10(38.4%) 11(44.0%) NS
Female	16(61.5%) 14(56.0%) NS
Duration of DM (years)	1.52±0.81 1.94±0.78 NS
Height (cm)	161.95±8.4 164.48±5.8 NS
Weight (Kg)	89.93±12.49 95.46±10.42 NS
BMI (Kg/m2)	34.35±4.73 35.21±3.27 NS
Waist circumference (cm)	103.71±11.25 108.19±9.96 NS
WHR	0.90±0.08 0.91±0.08 NS
WHtR	0.64±0.07 0.66±0.06 NS
FBG (mg/dL)	158.58±45.31 163.52±41.00 NS
Insulin (μIU/ml)	21.17±7.98 23.25±7.46 NS
HOMA-IR	8.28±3.61 9.38±3.48 NS
Leptin (ng/mL)	14.67±3.54 16.41±4.53 NS
Adiponectin (ng/mL)	20.40±9.20 16.86±8.41 NS
Creatinine (mg/dl)	0.77±0.12 0.79±0.11 NS

Data were expressed as mean ±SD, or number n (%) in percent of total; Students-test is statistically used to compare two independent means; NS: no significant differences (P>0.05). BMI, body mass index; WHR, waist to hip ratio; WHtR, waist to height ratio; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance.

Table 2: Comparison of anthropometric measurements and biochemical factors within (baseline and after 8 weeks) and between groups (n = 51).

Study variables	Fisetin Control Baseline After 8 weeks Baseline After 8 weeks
Weight (Kg)	89.93±12.49 85.41±11.19 *ab 95.46±10.42 93.80±10.14
BMI (Kg/m2)	34.35±4.73 32.62±3.46 *a 35.21±3.27 34.74±3.01
Waist circumference (cm)	103.71±11.25 100.37±10.55 **a 108.19±9.96 106.93±10.14
WHR	0.90±0.08 0.89±0.07 0.91±0.08 0.91±0.09
WHtR	0.64±0.07 0.62±0.06 0.66±0.06 0.65±0.07
FBG (mg/dL)	158.58±45.31 130.16±24.02**ab 163.52±41.00 155.24±39.02*
Insulin (µIU/ml)	21.17±7.98 16.97±5.12* 23.25±7.46 21.13±9.31
HOMA-IR	8.28±3.61 5.45±2.05*ab 9.38±3.48 8.12±3.61
Leptin (ng/mL)	14.67±3.54 11.99±3.03*ab 16.41±4.53 15.29±3.58
Adiponectin (ng/mL)	20.40±9.20 32.61±14.13**ab 16.86±8.41 20.66±10.57
Creatinine (mg/dl)	0.77±0.12 0.70±0.15 0.79±0.11 0.76±0.10

Data were expressed as mean ±SD; * significantly different (P≤0.05);** highly significant different (P≤0.01); paired t-test is statistically used to compare between baseline and after treatment results in the same group; Students-test is statistically used to compare two independent means; Mean values with (a) small letter were significantly different; (ab) small letters were highly significantly different between fisetin and control groups after 8 weeks.

7. Discussion

The results of the present study showed that body weight, BMI and WC decreased significantly, while no significant changes were observed in WHR and WHtR after 8 weeks in obese diabetic patients on fisetin supplement compared with those in the control group. These findings are in accordance with previous experimental study by Jung et al. (2013) who found that animal models fed a high fat diet (HFD) with fisetin considerably reduced weight gain, white and epididymal adipose tissue, and lost weight compared to those untreated mice (14). Fisetin treatment improved metabolic syndrome, reduced body weight, and inguinal fat pad weight in obese mice, according to Lin-Feng et al. (2019) (15). In HFD-induced obese mice, fisetin increased mRNA expression of thermogenesis and TCA-cycle-related genes in brawn adipose tissue and skeletal muscle, respectively, coupled with higher brown adipose tissue (BAT) weights, potentially leading to decreased adiposity (16). In the tissues of obese and HFD-fed animals, mammalian target of rapamycin (mTORC1) is highly active; in humans, obesity and insulin resistance increase the expression of the mTORC1 signaling effector p70 ribosomal S6 kinase (S6K) in visceral fat. High levels of branched-chain amino acids in obese or diabetic individuals may increase mTORC1 signaling through tuberous sclerosis protein 2 (TSC2) phosphorylation by Akt, which exacerbates lipid accumulation in adipocyte (17). Fisetin therapy, on the other hand, reduces Akt, mTORC1, and S6K1 phosphorylation in white adipose tissue (14). Another preliminary research reported that inhibition of mTORC1 increases thermogenesis and energy expenditure and prevents diet-induced obesity (10). As a result, inhibiting mTOR-S6K1 signaling might mitigate lipid accumulation, insulin resistance and attenuate obesity in individuals.

In discrepancy, the current study findings disagreed with previous findings that in HFD-induced mice treated with fisetin, Tomoaki et al. (2014) reported no anti-obesity effects were observed, such as weight loss and improvements in obesity-related blood biochemical markers and fatty liver (18). According to the literature review, there were no previous clinical studies to evaluate the effect of fisetin on anthropometric measurements to interpret these findings in humans.

The results of our study regarding FBG showed there was significant improvement in the fisetin group compared to the control group. These results are ccompatible with previous animal studies, since there has not been a previous clinical study evaluating the effect of fisetin on glycemic index in humans. According to Gopalan et al. (2014), Oral treatment of fisetin to diabetic rats resulted in a significant decrease in blood glucose and glycosylated hemoglobin levels (11). In an in vitro study, fisetin has been shown to inhibit glycogenolysis, gluconeogenesis and the formation of glycated hemoglobin (19). In accordance with another animal study, Jozaa Z. et al. (2021) suggested that fisetin reduced fasting hyperglycemia, glucose rises following oral and insulin tolerance tests, and HOMA-IR in diabetic rats (20). Reddemma et al. (2015), on the other hand, found no significant improvement in fasting plasma glucose following fisetin administration in diabetic rats (21). A number of studies have demonstrated that increased endogenous glucose synthesis is strongly linked to increased levels of two important gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase), raising the hypothesis that these enzymes may contribute to the development of diabetes (22). In obese type 2 diabetic patients, it

was found that a coordinated upregulation of gluconeogenic enzyme genes like PEPCK and G-6-Pase was associated with increases in insulin resistance, suggesting that obesity may latently enhance the gluconeogenic pathway even before fasting plasma glucose levels increase (23). Researchers believe that fisetin's anti-diabetic effects may be due to a variety of processes, including gene transcription in the liver tissue, which lowers the levels of PEPCK and G6Pase and also reduces the mRNA of PEPCK and G6Pase, indicating that the protein reduction is due to a drop in mRNA (11). Furthermore, supplementation with fisetin substantially enhanced the glucose-6-phosphatase/glucokinase ratio in obese mice, which reflects the delicate balance between hepatic glucose release and uptake. This ratio appears to be significantly lower in obese type 2 diabetic patients (16). In the present study, concerning the effect of fisetin on insulin levels and HOMA-IR, there was a significant reduction in HOMA-IR, but no significant change was observed in serum insulin levels after 8 weeks of supplementation in comparison to the control group, and this is in line with the study conducted by Sativa et al. (2021), where fisetin (10 mg/kg) oral given to diabetic rats for 42 days did not exhibit any noticeable effect on levels of serum insulin as compared to untreated diabetic rats (24). Meanwhile, study by Lin-Feng et al. (2019) showed that fisetin treatment lowered the HOMA-IR index in HFD-fed animals while attenuating the rise in blood glucose and insulin level (15). Furthermore, in obese, insulin resistant rats, treatment with fisetin reduced insulin resistance in the liver and increased peripheral insulin sensitivity (25). Myung et al. also reported that fisetin improved insulin resistance by correcting pancreatic islet dysfunction, which is known to increase insulin production per β-cell to compensate for insulin desensitization, and also reduced both hepatic gluconeogenesis and proinflammatory responses, both of which contribute to enhancing IR (16).

The results of this study showed that there was a significant increase in serum adiponectin and a significant decrease in serum leptin after fisetin administration compared with the control group at the end of the study. These results are in agreement with a previous study conducted by Chian et al. (2018), who found that obese mice administered fisetin intraperitoneal at 20 mg/kg for 10 weeks had significantly lower blood leptin levels than obese mice not administered the drug (26). Another study revealed that fisetin-supplemented HFD-fed animals had significantly higher plasma adiponectin levels and a lower leptin/adiponectin ratio after 16 weeks (16). Previous research has shown that fisetin can increase adiponectin levels in 3T3-L1 adipocytes via increasing Sirtuin 1 (sirt1) expression and suppressing mTORC1 signaling to prevent 3T3-L1 preadipocyte differentiation (14). The decreased adiponectin gene expression in obesity and type 2 diabetes may be due to decreased Forkhead box protein O1 (Foxo1) a negative regulator of adipogenesis and sirt1 expression and reduced Foxo1-C/EBP-α transcription complex formation. Thus, adiponectin gene expression in differentiated 3T3-L1 adipocytes was boosted by overexpression of Foxo1. In addition, SIRT1 boosted Foxo1 and CCAAT/enhancer binding proteins-α (C/EBP-α) mediated adiponectin promoter activation and regulated the development of the Foxo1-C/EBP-α transcriptional complex (27). In obese individuals, Stefania et al. (2020) discovered a parallel but opposing behavior of SIRT1, leptin, and adiponectin. SIRT1 had a positive association with adiponectin and a negative association with leptin, both of which were consistent with fat mass and distribution. When extra adipose tissue is present, low SIRT1 levels are seen in the face of high leptin levels, which might be a contributing cause to the persistent resistance to leptin response seen in people with obesity (28). SIRT1 activation boosts peripheral and central leptin signaling, overcoming diet- and obesity-induced leptin resistance (28). No previous clinical studies about the effect of fisetin on adiponectin and leptin levels are available to interpret these findings in humans.

The results of the current study showed that there was no significant decrease in serum creatinine after 8 weeks of fisetin administration compared with the control group. No available clinical trials evaluate the effect of fisetin on serum creatinine levels. The current findings are consistent with those of Savita et al. (2021), who found that fisetin at a low dosage (2.5 mg/kg) had no noticeable effect on kidney function markers in diabetic rats (24). Xu Sun et al. (2018) also reported similar findings among tumor-bearing mice (29). On the contrary, an earlier study in mice with acute renal damage has been shown to find that fisetin decreases serum creatinine and blood urea nitrogen levels, indicating that it can attenuate aberrant kidney function (30).

8. Conclusions

The current study showed that fisetin supplementation can attenuates obesity and improve the diabetic status in obese diabetic patients. Therefore, its application as a complementary treatment, along with other common treatments in obese diabetic patients can be suggested. However, future clinical investigations seem necessary to determine its other effects in obesity.

9. Conflicts of interest

There are no conflicts of interest to declare.

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