Effect of bromelain in obese diabetic patients in Iraq

Efecto de la bromelina en pacientes diabéticos obesos en Irak

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Abstract

Background: In vitro and in vivo studies, bromelain has been found to reduce obesity. Objective: Obese patients with type 2 diabetes were studied to evaluate if a dietary supplement (bromelain) might help them lose weight. Methods: The effects of 8 weeks of bromelain on anthropometric parameters, HOMA-IR, and blood levels of fasting glucose, leptin, IL-6, and TNF-α were studied in this randomized, single-blind, controlled trial conducted in Iraq. 52 obese diabetic patients from Iraq (25 men and 27 women) were randomly assigned to one of two groups: bromelain or control. During an 8-week study, the bromelain group was given bromelain capsules (500 mg twice daily) in addition to their prior medication, metformin tablets (500 mg three times daily), whereas the control group received metformin tablets (500 mg three times daily). Results: In the bromelain group, there was a substantial reduction in BMI, WC, WHR, HOMA-IR, serum leptin, IL-6, and TNF-α levels after the study compared to the baseline. The bromelain group exhibited a significant difference in BMI, WC, HOMA-IR, leptin, IL-6, and TNF-α blood levels when compared to the control group. The blood levels of fasting plasma glucose (FBG) and waist-hip ratio (WHR) did not change compared to the control group. According to the findings, bromelain may help in the treatment of obesity, improve insulin sensitivity, and exhibit an anti-inflammatory effect in obese diabetic patients, implying that it may be a potential supplemental anti-obesity medication for these individuals who warrant further research.

Keywords: bromelain, obesity, type2 diabetes, leptin, IL-6, TNF-α.

Resumen

Antecedentes: En estudios in vitro e in vivo, se ha descubierto que la bromelina reduce la obesidad. Objetivo: Se estudiaron pacientes obesos con diabetes tipo 2 para evaluar si un suplemento dietético (bromelina) podría ayudarlos a perder peso. Métodos: Los efectos de 8 semanas de bromelina sobre los parámetros antropométricos, HOMA-IR y los niveles sanguíneos de glucosa en ayunas, leptina, IL-6 y TNF-α se estudiaron en este ensayo aleatorizado, simple ciego y controlado realizado en Irak. 52 pacientes diabéticos obesos de Irak (25 hombres y 27 mujeres) fueron asignados aleatoriamente a uno de dos grupos: bromelina o control. Durante un estudio de 8 semanas, el grupo de bromelina recibió cápsulas de bromelina (500 mg dos veces al día) además de su medicación anterior, tabletas de metformina (500 mg tres veces al día), mientras que el grupo de control recibió tabletas metformina (500 mg tres veces al día). Resultados: En el grupo de bromelina, se observó una reducción en los niveles de IMC, WC, WHR, HOMA-IR, leptina sérica, IL-6 y TNF-α al finalizar el estudio en comparación con la línea de base. El grupo de bromelina mostró una diferencia significativa en los niveles sanguíneos de IMC, CC, HOMA-IR, leptina, IL-6 y TNF-α en comparación con el grupo de control. Los niveles de glucosa plasmática en ayunas (FBG) y y de la relación cintura cadera (WHR) no cambiaron de manera estadísticamente significativa en comparación con el grupo de control. Según los hallazgos, la bromelina puede ayudar en el tratamiento de la obesidad, mejorar la sensibilidad a la insulina y exhibir un efecto antiinflamatorio en pacientes diabéticos obesos, lo que implica que puede ser un medicamento complementario potencial contra la obesidad para estas personas que justifica una mayor investigación.

Palabras clave: bromelina, obesidad, diabetes tipo 2, leptina, IL-6, TNF-α.

Introduction

Obesity has become a prominent worldwide health problem in the last century because of recent environmental and sociological changes that encourage positive energy balances and weight gain. Consumption of high-calorie or high-fat meals, lack of physical exercise, and a change toward a well-developed sedentary lifestyle are the key reasons[1]. Numerous clinical investigations have found a link between obesity and insulin resistance in adults and children, as well as evidence that weight loss is related to a drop in insulin concentration and an improvement in insulin sensitivity. Obesity is a frequent co-morbid condition in people with type 2 diabetes and is commonly referred to as "Diabesity."[2]. Given the high prevalence of type 2 diabetes and obesity, novel therapeutic strategies targeting shared pathomechanisms are urgently needed[3]. Various anti-obesity drugs have been tested in clinical studies. However, along with their anti-obesity effectiveness, these drugs might have unexpected side effects. Due to these side effects, there is a high demand for effective yet safe anti-obesity drugs. Thus, several medicinal plants and their derivatives may work as natural anti-obesity drugs with minimal or no side effects. Plant-derived natural compounds contain anti-inflammatory and antioxidant effects. When used as dietary supplements, these compounds often help with weight loss and other issues[4]. Pineapple (Ananas comosus) is the most popular edible member of the Bromeliaceae family, and it is produced in a variety of tropical and subtropical nations such as the Philippines, Thailand, Indonesia, Malaysia, Kenya, India, and China[5]. It has been utilized in numerous native cultures as a medicinal plant and these therapeutic characteristics are ascribed to bromelain, a crude pineapple extract which, among other substances, contains several proteinases that are closely related[6]. Stem bromelain (SBM), a phytotherapeutic protein, is an anti-obesity alternative therapy. According to a previous in vitro study, SBM reduces adipogenic gene expression in 3T3-L1 adipocytes and causes apoptosis and lipolysis in mature adipocytes. SBM inhibited adipogenesis by decreasing CCAAT/enhancer binding proteins (C/EBP) and peroxisome proliferator-activated receptor-gamma (PPAR-γ) expression independently of C/EBP gene expression[7]. In addition, according to Weidong Xie et al. (2006), ananas comosus improved insulin sensitivity in type 2 diabetic rats, whereas ananas comosus may one day be utilized to treat diabetics with insulin resistance[8]. Furthermore, the increased release of adipocytokines and pro-inflammatory cytokines is caused by obesity-induced adipose tissue dysfunction, leading to insulin resistance[9]. Bromelain may also act to modulate inflammation and other factors that are connected to the pathogenesis of cardiovascular disease and health problems in people with diabetes[10]. Bromelain has been shown previously to have an immunomodulatory impact. Bromelain increases granulocyte-macrophage colony-stimulating factor, interleukins, IL-2, and IL-6, and decreases T-helper cell activation. As a result, bromelain is an effective anti-inflammatory agent in a variety of different situations[6]. Even though several laboratory and animal studies have proven bromelain to be anti-obesity and can improve diabetic state, there has been no clinical research to assess the influence of bromelain as a natural dietary ingredient on obesity and diabetes. This study examined the impact of bromelain supplementation on obesity and related variables in obese type 2 diabetics.

Patients and methods

The effects of 8 weeks of bromelain (1000 mg/d) supplementation on obese patients with type 2 diabetes were evaluated in this randomized, single-blind, controlled trial. The study was performed in Baghdad, Iraq, between October 2020 and December 2021 and was approved by the College of Pharmacy/AL-Mustansiriyah University's local ethics committee as well as the Ministry of Health. During their visit to the National Diabetes Center for Treatment and Research/Al-Mustansiriyah University, 60 obese diabetic patients were recruited for the research. The trial was only completed by 52 patients (27 female and 25 males). All participants were given a thorough explanation of the study's purpose before giving their informed permission. The main inclusion criteria were indeed a BMI of ≥30 kg/m^2 and a waist circumference of greater than 88 cm for women and greater than 102 cm for men, as well as having type 2 diabetes mellitus and being between the ages of 18 and 50. Patients with considerable health issues such as renal illness, cardiovascular disease, liver disease, uncontrolled thyroid function, diabetes complications, or other chronic health concerns were also excluded. Other exclusion criteria involve patients who were already taking hypoglycemic drugs other than metformin; patients who were already taking bromelain supplements or other herbal supplements; history of allergic reactions to bromelain and bee stings or olive tree pollen; and pineapple; and patients who had previously taken anti-obesity drugs before enrollment; and inability to provide informed consent, i.e., unwillingness to participate in the study procedure.

Randomization and intervention

The trial included an 8-week treatment period. The patients were randomly assigned to either the bromelain or the control groups. The bromelain group included 27 patients who received 500 mg of bromelain (manufactured by Now Foods/USA) twice a day in addition to their regular medication (500 mg of metformin three times per day). The control group included 25 patients who were currently taking 500 mg of metformin three times per day. Kelly[11] stated that the best action of bromelain occurs

at a dose of 750–1000 mg/d over a longer period of time, hence the 1000 mg dosage was chosen. Randomization with the assignment was disguised in this investigation by using opaque prenumbered envelopes. Compliance was tested at the end of the eight weeks by counting the returned capsules. Patients were called once a week to ensure that they were taking the capsules as prescribed.

Measurements

Anthropometric Measurements

Patients were dressed in light indoor clothing and had their body weight assessed using a 0.5 kg precision weighing scale. Standing height was measured without shoes on a flat platform with weight evenly distributed on both feet and heels together using a stadiometer to the nearest 0.5 cm. A vertical board with a metric rule attached and a horizontal headboard that may meet the highest point on the head have been used to measure height. By dividing one's weight in kilograms by the square of one's height in meters (kg/m^2), the BMI was calculated. The waist circumference was measured midway between the bottom border of the last noticeable rib and the top of the iliac crest, to the nearest 0.5 cm. The patient stood with her feet close together, her arms on the sides, and her body weight evenly distributed while wearing light clothing. The waist-hip ratio (WHR) was calculated by dividing the waist circumference by the hip circumference.

Other Blood Test and Biochemical Data Measurement

All blood tests and other biochemical data were gathered and examined in the standard laboratory at Al-Mustansiriyah University's National Diabetes Center for Treatment and Research before and after the 8-week treatment. Blood samples were taken in the morning after a 12-hour fast. Blood glucose levels were determined using the glucose oxidase method. Serum leptin, IL-6 as well as TNF-α, were measured using Sandwich-Enzyme-Linked Immunosorbent Assay (ELISA) kits (Demeditec, Germany) and (Cusabio, China), respectively. To compare differences in insulin resistance profiles, the HOMA technique was utilized (HOMA-IR). HOMA-IR = (Fasting serum insulin μIU/mL)* (Fasting blood glucose mg/dl)/405.

Statistical analysis

The accessible statistical package SPSS-27 was used to analyze the data (Statistical Packages for Social Sciences-version 27). Simple measurements of mean and standard deviation were used to present the data. The significance of the difference between various means (quantitative data) was assessed using the Students' t-test for differences between two independent means and the Paired-t-test for differences between paired observations (or two dependent means). To compare categorical variables, the chi-squared test was utilized. When the P value was equal to or less than 0.05, it was considered statistically significant.

This research included a total of sixty patients. For unclear reasons, five patients in the control group withdrew from the research, while three patients in the bromelain group were eliminated. Two of the three patients were unable to finish the trial owing to ineffectiveness, while the other one was unable to comply. As a result, 52 individuals were evaluated for the final analysis. The mean ± standard deviation of participants' ages was 40.73±9.6 and 38.62±7.3 years, 51.9%were male and 48.1% female in the bromelain group, whereas 44.0% were male and 56.0% female in the control group. Table 1 shows the demographics and characteristics of patients in the bromelain and control groups. At baseline, there were no significant differences between the two groups in terms of demographics or patient characteristics.

Table 2 summarizes the differences in anthropometric measurements, HOMA-IR, and serum levels of fasting blood glucose, leptin, IL-6, and TNF-α at baseline and after 8 weeks and between the bromelain and control groups.

The within-groups (at baseline and after 8 weeks) analysis showed that there was a significant decrease in BMI ($p \leq 0.01$) from 35.18±3.81 kg/m^2 to 33.04±2.76 kg/m^2, WC ($p \leq 0.01$) from 105.87±9.29 cm to 101.07±9.17 cm, WHR ($p \leq 0.05$) from 0.90±0.07 to 0.88±0.06, HOMA-IR ($p \leq 0.01$) from 7.98±3.16 to 5.47±1.94, serum levels of FBG ($p \leq 0.05$) from 166.22±37.36 to 149.56±31.49, leptin ($p \leq 0.01$) from 17.26±4.24 ng/mL to 13.02±3.04 ng/ml, IL-6 ($p \leq 0.01$) from 24.80±8.61 to 13.86±4.36 pg/mL and TNF-α ($p \leq 0.01$) from 52.63±10.34 to 36.25±8.09 pg/mL in the bromelain group at the end of the study in comparison with the baseline. There were significant changes ($p \leq 0.05$) during the study in the serum levels of glucose in the control group. Additionally, there were no significant changes ($P > 0.05$) in the anthropometric parameters and other assessed biochemical factors in the control group. Between bromelain and control groups comparison of changes in the anthropometric parameters, biochemical factors, and adipokines showed a significant difference in BMI ($p \leq 0.05$), WC ($p \leq 0.05$), HOMA-IR ($p \leq 0.01$), serum levels of leptin ($p \leq 0.05$), IL-6 ($p \leq 0.01$) and TNF-α ($p \leq 0.01$), but no significant changes ($P > 0.05$) in WHR and serum levels of FBG.

Table 1. Baseline demographic and characteristics of the patients (N = 52)

Study variables	Bromelain	Control	p-value
Age (years)	40.73±9.6	38.62±7.3	NS
N	27	25	NS
Gender N (%)			
Male	14(51.9%)	11(44.0%)	NS
Female	13(48.1%)	14(56.0%)	NS
Duration of DM (years)	1.44±1.23	1.94±0.78	NS
Height (cm)	163.42±7.8	164.48±5.8	NS
BMI (Kg/m^2)	35.18±3.81	35.21±3.27	NS
Waist circumference (cm)	105.87±9.29	108.19±9.96	NS
WHR	0.90±0.07	0.91±0.08	NS
FBG (mg/dL)	166.22±37.36	163.52±41.00	NS
HOMA-IR	7.98±3.16	9.38±3.48	NS
Leptin (ng/mL)	17.26±4.24	16.41±4.53	NS
IL-6 (pg/mL)	24.80±8.61	30.34±11.85	NS
TNF-α (pg/mL)	52.63±10.34	47.96±6.88	NS

Data presented as mean ± standard deviation (SD), or number N (%) in percent of the total; When comparing two independent means, the Students-test is employed statistically; NS: No statistically significant differences (P>0.05). BMI=body mass index; WHR= waist to hip ratio; FBG= fasting blood glucose; HOMA-IR= homeostasis model assessment of insulin resistance; IL-6=Interleukin-6; TNF-α= tumor necrosis factor-alpha.

Table 2. Comparison of anthropometric parameters and biochemical factors within (baseline and after 8 weeks) and between groups (N = 52).

Study variables	Baseline	Bromelain After 8 weeks	Baseline	Control After 8 weeks
BMI (Kg/m^2)	35.18±3.81	33.04±2.76**a	35.21±3.27	34.74±3.01
Waist circumference (cm)	105.87±9.29	101.07±9.17**a	108.19±9.96	106.93±10.14
WHR	0.90±0.07	0.88±0.06*	0.91±0.08	0.91±0.08
FBG (mg/dL)	166.22±37.36	149.56±31.49*	163.52±41.00	155.24±39.02*
HOMA-IR	7.98±3.16	5.47±1.94**ab	9.38±3.48	8.12±3.61
Leptin (ng/mL)	17.26±4.24	13.02±3.04**a	16.41±4.53	15.29±3.58
IL-6 (pg/mL)	24.80±8.61	13.86±4.36**ab	30.34±11.85	27.88±9.22
TNF-α (pg/mL)	52.63±10.34	36.25±8.09**ab	47.96±6.88	45.59±8.97

Data presented as mean ± SD; baseline versus after 8 weeks: *(P≤0.05); **(P≤0.01); The paired t-test is a statistical method for comparing baseline and after 8 weeks of treatment outcomes in the same group; when comparing two independent means, the Students-test is utilized; bromelain versus control after 8 weeks: a (P≤0.05); ab (P≤0.01).

Discussion

The present study indicated that the patients on bromelain supplement showed a significant reduction in BMI, and waist circumference, while no significant changes were observed in WHR compared with those in the control group. The results of the present study are in line with previous experimental studies since there has been no previous clinical research to investigate the influence of bromelain on anthropometric parameters. Using 50 µg/mL proteolytically active stem bromelain as a therapy for 3T3-L1 pre-adipocytes for eight days, Sandeep et al. (2012) found that adipogenic gene expression was decreased, apoptosis was induced, and lipolysis was seen in mature adipocytes[7]. Protein kinase B (Akt) enhances cell survival by regulating cellular growth factors and preventing apoptosis by deactivating pro-apoptotic proteins[12]. Because bromelain reduces adipocyte viability and cell counts by inhibiting Akt activity and promoting apoptosis, one mechanism through which it has anti-obesity actions is that it decreases the survival of mature adipocytes. Moreover, bromelain inhibits lipolytic genes while increasing lipolysis[7,13]. In addition, prior research has shown that pineapple leaf extract and dried pineapple cuts have a substantial influence on weight loss in hypercholesterolemic animals and suggest that the bromelain component of pineapple is responsible for this action[14]. Po-An et al. (2020) found that daily treatment of mice with high-fat diet bromelain (20 mg/kg) for 12 weeks decreased high-fat diet (HFD)-induced obesity and deregulated adiposity in white adipose tissue (WAT) and brown adipose tissue (BAT) whiting[15]. It has been found

that animals with a higher BAT level are more resistant to obesity and type 2 diabetes. Fat oxidation and total body energy expenditure are both increased by human BAT, the same as mouse BAT. According to a previous study, obesity has been linked to a decrease in BAT function and activity. Obesity and type 2 diabetes are both associated with decreased glucose uptake in BAT[16]. Bromelain may thus be a good match for raising total energy expenditure and reducing obesity and related metabolic illnesses in humans.

In this study, there was no significant improvement in blood glucose levels after 8 weeks of supplementation with bromelain compared to control, and these findings were consistent with other investigations. People with type 2 diabetes who took bromelain for 12 weeks had neither improvement nor worsening in their blood sugar management, according to Chit Moy et al. (2016)[17]. Pineapple ethanol extract reduced fasting blood glucose levels in diabetic rats, but this effect was not significantly different from that of metformin or a negative control[18]. On the contrary, a study by Muhammad et al. (2020) demonstrated that diabetic rats treated with ananas comosus leaf extract had significantly lower blood glucose levels[19]. No previous clinical study has examined the effects of bromelain on HOMA-IR, but the findings of this study showed that it had substantial effects on HOMA-IR when compared to controls. The current findings agree with those reported that exogenous insulin sensitivity was enhanced, and insulin resistance was prevented in diabetic dyslipidemic rats given ethanolic extracts of ananas comosus leaves, according to earlier research[8]. There was also an improvement in insulin sensitivity in HepG2 cells. This is similar to what happened in rats, and it suggests that more research is needed to figure out the exact mechanism behind the change[8]. It is known that Peroxisome proliferator- activated receptor gamma (PPAR-γ) expression is inhibited by bromelain. It is that this influences weight reduction and thus might have a role in regulating insulin sensitivity in obese diabetes patients. As fat and free fatty acid levels rise as a result of homeostatic changes, insulin resistance and hyperinsulinemia may develop[20]. Numerous clinical studies have found that reducing body weight can reduce insulin resistance and insulin concentration, as well as the size of adipocytes, which improves fat storage and, as a result, reduces the distribution of free fatty acids from fat cells to muscle tissue and the liver in obese individuals[2,21].

The current investigation found that after 8 weeks of bromelain treatment, serum leptin levels were significantly lower than in the control group. The findings of this study are consistent with earlier animal findings. Pineapple juice has been shown to diminish HFD-induced hyperleptinemia in rats, and the decrease in visceral fat in obese rats fed pineapple juice may explain the lower serum levels of leptin in those animals[22]. However, taking bromelain daily for 12 weeks increased leptin levels in WAT in rats on a high fat diet[15]. For more than a decade, it has been recognized that fatty tissues create several biological molecules like leptin and other cytokines known as adipokines that contribute to regulating insulin sensitivity in the peripheral tissues[23]. Increases in body fat mass are associated with higher blood levels of the hormone leptin, which lends credence to theories that the white adipocytes seen in this tissue are a major source of leptin[24]. The activation of PI3K/AKT and mTOR is required for the induction of adipogenesis, which leads to PPARγ activation, increases in body fat mass, and subsequent leptin synthesis[25-28]. In adipocytes, stem bromelain appears to inhibit Akt phosphorylation, PPARγ expression, and adipogenesis[7]. These events might be linked to decreased adipose tissue mass and hence a decline in leptin synthesis. This is the first study to look at how bromelain affects leptin levels in a clinical setting. This means that our findings can be seen in the light of that. A lot of macronutrients in the adipose tissues facilitates inflammatory mediators like TNF-α and IL-6 to be released, which leads to a more inflammatory state in the body[29-32]. In this regard, the current study found that when compared to the control group, bromelain significantly reduced fasting serum levels of IL-6 and TNF-α in obese diabetic patients after 8 weeks. There had been no previous clinical trials to investigate the effect of bromelain on blood levels of these pro-inflammatory markers, so these results are compatible with experimental research by Orapin et al. (2021) who demonstrated that pre-treatment of RAW 264.7 macrophage cells with bromelain inhibited lipopolysaccharide (LPS)-induced IL-6 and TNF-α production by suppressing NF-$_k$B activation and downregulating mitogen-activated protein kinases (MAPK) pathway proteins such as extracellular signal-regulated protein kinase (ERK1/2), c-Jun N-terminal Kinase (JNK), and p38[27]. In human U937 macrophages, Thitima et al. (2018) found that co-treatment with LPS and bromelain suppressed IL-6 gene expression[28]. In addition, Siavash et al. (2020) found that bromelain-treated inflammatory tissue from people with inflammatory bowel disease had less TNF- α and IFN-γ expression[29]. This study's findings, on the other hand, contradict previous research that found bromelain promotes the production of IL-1, IL-6, INF-γ, and TNF-α in mouse macrophages and human peripheral blood mononuclear cells[6].

Conclusions

According to the current study, bromelain supplementation can reduce obesity, increase insulin sensitivity, and have an anti-inflammatory impact on obese diabetic patients. As a result, its use in obese diabetic patients as a supplemental therapy to other popular therapies is advised. Future clinical trials, however, appear to be required to assess its additional benefits for obesity.

Conflicts of interest

There are no potential conflicts of interest.

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