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Anti-obesity effect of peel of *Opuntia joconostle* Web in high-fat-diet-induced obesity C57BL/6J mice

Research Article

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Abstract

Obesity is directly associate to cancer, diabetes, cardiovascular diseases and hypertension producing severe complication of health and economic costs in a global scale. This study aims to validate the anti-obesity potential of the peel of *Opuntia joconostle* hydroethanolic extract (XOCO) on obesity in high-fat diet (HFD)-induced obese C57BL/6 mice. Therefore, the phenolic compounds and flavonoids profiles were investigated by colorimetric assay. We are using a HFD induced obesity in mice and after orally administered 200, 300 and mg/kg XOC for 5 weeks. At the end of the experimental period was determined body weight, food intake, serum biochemical parameters, fat weight, organ and a histological analysis. This research demonstrated that the peel of the investigated xoconostle had a significantly amount of flavonoids, showing highest level of total phenolic compound (58.15 mg GAE/g of extract) and flavonoid content (59.12 mg CE/g of extract). XOC Treatment in HFD-fed mice decreases adipogenesis, total body fat, the absolute body weight, lipidemia, glucose levels, leptin, and resistin, and increases HDL-CHO levels and adiponectin. In histological analysis showed inhibition of the deposition of fat droplets in the liver. Findings indicated that XOC has anti-obesity effects in obesity-induced mice. This potential anti-obesity effect may be due to phenolic compounds content in the extract.

Key Words: *Opuntia joconostle* Web., Obesity, Flavonoids, Phenolic compounds.

Introduction

Obesity is associated with the accumulation of excess body and modification of lipid metabolism due to the storage of excess energy in adipocytes produced by high food intake. It is characterized by the disequilibrium of the adipose tissue brown (BAT) and white (WAT). This disorder is a public health problem worldwide associated with metabolic diseases, including hypercholesterolemia, type 2 diabetes, and cardiovascular disease. In consequence is vital to develop methods for preventing obesity in more people such as natural inhibitors of absorption and digestion of lipids content in the diet thus reducing the energy intake throughout gastrointestinal mechanisms without modified these mechanisms (1).

Hyperlipidemia is characterized by an increase in serum low-density lipoprotein and a decrease in high-density lipoprotein levels. Consuming high-fat diets induces hyperlipidemia and an obesity-related increase in cholesterol and triacylglycerol in tissues and plasma (2). Changes in lipid metabolism are generated by the disturbance in the liver, such as high access of lipids, default in the function and/or structure of lipoproteins, lower mobilization of fat, which consequently induces hepatomegaly, accumulation of lipids in the liver, modifications in the color and shape of the liver (3).

Previous reports indicated that anti-obesity drugs have high toxicity and adverse effects. The use of drugs to treat obesity can rebound weight gain and lead to drug abuse. The drugs widely used as anti-obesity agents include fluoxetine, diethylpropion, phentermine, orlistat, sibutramine, and bupropion. Among them, diethylpropion and phentermine are only approved for short-term treatment because there is a high potential for them to be abused (4). Meanwhile, sibutramine and orlistat, due to their low toxicity, are approved for longer periods in the treatment of obesity. Surgical treatment interventions used as bariatric surgery are not always suitable. Nowadays, these treatments do not display effectiveness in producing prolonged long-term weight loss. As obesity is considered a chronic medical disease, more strategies that focus on the need to develop new drug therapies to decrease the predominance of obesity are being explored (5).

Medicinal herbs containing bioactive phytochemicals become important for human health with few side effects. Consequently, a great variety of plants have been used as traditional medicines for treatment numerous diseases (6).

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In Mexico like in other countries traditional medicine is used as therapy in the traditional medical systems. One hundred thirty-nine plant species, belonging to sixty-one families, native to Mexico, have been reported to be used in Traditional Mexican Herbal Medicine for the treatment of obesity (7). Among the plants used in the treatment of obesity is the fruit of *Opuntia joconostle* F.A.C Weber ex Diguet commonly known as Xoconostle is an Ayurvedic fruit used to cure diabetes and obesity main (8). *O. joconostle* seeds methanolic extract showed a significant inhibition in lipid levels in hypercholesterolemic mice (9). Therefore, the aim of this investigation was to evaluate the peel of *O. joconostle* extract in high-fat-diet (HFD)-induced obese C57BL/6 mice with the purpose of searching newly safe and potent natural agents for the therapy of obesity.

Materials and Methods

Plants materials

Fruit of *O. joconostle* was collected in the state of Hidalgo and authenticated in the herbarium of Escuela Nacional de Ciencias Biologicas-IPN and registered with herbarium number: ENCB-57643, An electric grinder was used to grind the dried peel to a particle size that passed through a 0.5 mm sieve.

Preparation of the extract

Powdered air-dried peel of *O. joconostle* (300 g) was subjected to ultrasound extraction with an extraction time of 30 min, ethanol concentration of 80%, frequency 40KHZ and power 90 N. The hydroethanolic extract was then reduced in a rotary vacuum evaporator to yield crude extract.

Flavonoid Content

To estimate the flavonoid amount, 4 mL of distilled water were mixed to plant extract (1 mL), $NaNO_2$ solution (5%; 0.3 mL), aluminum chloride ($ALCl_3$) solution (10%; 0.5 mL) and stirred for 10 min. Then the tubes were incubated at 25 °C for 5 min, immediately reading at λ 510 nm. Flavonoid content was expressed as (+)-Catechin equivalent per 100 grams dry weight using a calibration plot with (+)-Catechin (10).

Determination of the total phenolic compound (TPC)

The measure of TPC was performed by the Folin–Ciocalteu colorimetric assay (11). 1.5 mL of Folin–Ciocalteu reagent (20% v/v) and 0.2 mL of each extract were mixed in-depth. Then 4 mL of Na_2CO_3 (7%) were incorporated, then made up to 10 mL with water. The solution was kept at room temperature in the dark for 90 min. The absorbance was then determined at 760 nm using a UV-spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). TPC was calculated using a calibration curve of gallic acid.

Animals of experimentation

C57BL6/J mice (males) six weeks old and 24-29 g of body weight were used in this research. The mice were housed in groups of eight in standard plastic rodent cages with laboratory conditions (12 h light/dark cycle), temperatures 20–22◦C, and were fed with normal chow (Purina), and water ad libitum. In addition, all animal experiments were approved by the Animal Ethics Committee of Escuela Nacional de Ciencias Biológicas, IPN (Code: Folio ENCB/CEI/ 046/2021), in accordance with the principles of the Committee on Care and Use of Laboratory Animals (NIH publications 85-23, revised 1985) and comply with Mexican Official Normativity (NOM -062-Z00-1999).

Experimental Procedure

Induction of obesity

The high-fat diet used in this experiment was prepared with 140 g of casein, 1.8 g of L- cysteine, 120 g of animal butter, 40 g of soybean oil, 150 g of maltodextrin 10, 450 g of sucrose, 50 g of cellulose, vitamins, and minerals (tablet equivalent) and 2.5 g of choline bitartrate (12). The groups formed (n = 8) was subjected to different treatments: Group (1) normal diet; Group (2) high-fat diet (HFD); Group (3) HFD + 200 mg/kg of XOCO; Group (4) HFD + 300 mg/kg of XOCO; Group (5) HFD + 400 mg/kg of XOCO; Group (6) HFD + 30 mg/kg of phentermine. The extracts and phentermine (13) were given daily through a feeding bottle. All the groups were fed for five weeks, and the amount of food consumed was registered daily.

The obesity in mice was monitored by evaluating food intake and body weight increase in the obese animals. Both food intake and body weight were measured once per week. At the end of the 5-week period (12), the mice were fasted overnight, after which they were sacrificed by cervical dislocation and necropsied. Fresh liver, kidney, and spleen were removed surgically and weighed. Adipose tissue (WAT) was removed surgically and weighed. In addition, blood samples were taken from the inferior vena cava and centrifuged at 4000 r/min for 10 min TG, TC-CHO, HDL-CHO, adiponectin, leptin, resistin, serum hepatic marker enzymes aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using commercial assay kits according to the manufacturer's instructions (Sigma-Aldrich, St Louis MI, USA).

Adipose tissue and liver were fixed in paraformaldehyde (4%) and then embedded in paraffin. These tissues were cut at 3 μm, and the largest slides were stained with hematoxylin-eosin (HE). Slides were recorded in a light microscope, and adipocyte size was measured using MetaMorph software (Molecular Devices, PA, USA).

Statistical analysis

All experimental results are mean ± standard deviation of three independent assays. Standard error was calculated with a one-way ANOVA analysis of variance using IBM® SPSS 16.0 (SPSS, Inc, Chicago, IL), followed by Tukey significant difference. $P< 0.05$ was considered statistically significant.

Results and Discussion

The varieties of xoconostle fruits are rich in flavonoids and phenolic compounds and have been traditionally used for enhancing human health due to their bioactive effects (14). The XOC extract showed the highest level of flavonoid content (59.12 mg CE/g of extract) and total phenolic compound (58.15 mg GAE/g of extract).Therefore, these compounds may be responsible for the anti-obesity effect of this fruit. in this investigation, we established a model of obesity by feeding mice with a high-fat diet (HFD) and assayed the anti-obesity activity of the XOC extract.

The animal groups were given a high-fat diet during the 5 weeks significantly ($P< 0.05$) gained weight compared to the normal fat diet group (ND), supporting that obesity was induced in the time-proposed model, indicating that the obesity model was successful. In mice, the changes in their body weight with the administration of the extract at 200, 300 and 400 mg/kg for 5 weeks are shown in Table 1. The percentages of weight reduction compared to the HFD group in groups HFD+XOC at 200, 300 and 400 mg/kg were 32.3%, 48.6%, and 52.2% respectively. These results demonstrate that XOC at a dose of 400 mg/kg is more effective than phentermine (30 mg/kg) reduction of body weight gain.

Food intake was evaluated to observe changes in food efficiency ratio and appetite. The average food intake in the HFD + XOC (400 mg/mL) group at the end of the experiment was 35% lower compared with the obese group (HFD), showing an average of 1.4 g reduction in food intake compared with the obese group (Table 1). In the HFD-fed mice, food intake was higher by 1.5-fold than those in the normal control; food intake significantly ($p<0.05$) reduced in all treated groups in a range of 12.5% to 35%. The diet efficiency rate in the HFD group was 16%, while in the control group (ND) was 11%.

High-fat dietary intake produces insulin resistance altering lipid and glucose metabolism. Obesity status was evaluated by determining food intake and body weight because these parameters are key in treating obesity. Considering that food intake is usually higher in the obese state, food intake and body weight were measured once per week. This difference suggested that XOC can suppress HF-induced body weight gain. We report for the first time that the effect of oral supplementation of herbal formulation using peel of *O. joconostle* (XOC) in obese mice induced by a High-Fat diet for 5 weeks resulted in a considerable reduction in weight gain in these groups. The treatment of XOC (400 mg/kg/day) produced a decrease in food intake and consequently a reduction in body weight. These results suggest that the decrease in body weight with XOC treatment was, in part, caused by a reduced food intake. This reduction in food intake carries out low energy excess and low caloric intake, consequently reducing adiposity. Thus, the reduction in food intake can be understood as a decline in appetite. In addition, it also may be due to decreasing body weight by suppressing the expansion of adipose tissue mass.

The levels of lipidemia after 5 weeks of treatment are shown in Table 1. Findings showed that the high-fat diet significantly increased serum LDL-CHO, TC-CHO, TG, and NEFA compared to the ND group ($p<0.05$), which were significantly elevated by 2.92-fold, 3.59-fold, 2.08-fold, and 1.4-fold respectively compared to those in the ND groups and a significantly ($p< 0.05$) lower level of serum HDL (high-density lipoprotein) in 34.2% compared to those in the ND groups demonstrating that the obesity status caused hyperlipidemia. The increased levels of plasma of these parameters in obese mice were significantly improved by the supplementation of 400 mg/kg/day XOC extract by 11.6%, 44.4%, 50.6%, 49%, and 26.5%, respectively, compared to the control group (HFD). While HDL increases show no significant difference from the control group.

High levels of total cholesterol increase the risk of atherosclerotic and cardiovascular diseases. Thus, the treatment of dyslipidemia is very important in preventing cardiovascular disorders (15). In our study, HDL-CHO, LDL-CHO, TC-CHO, TG, and NEFA were lower in the XOC group compared to those in HFD and phentermine groups. Our results displayed that the HFD group exhibited an increase in fat content, mainly in the liver and abdominal tissues, which were significantly inhibited after the XOC extract treatment. The results indicated that herbal formulation could reduce hyperlipidemia and its complications.

The effect of XOC on indicators of obesity and metabolic syndrome, such as the weight of adipose tissue, was evaluated. Adipose tissues, including white adipose tissue as subcutaneous and visceral (adipose tissue white (WAT)), were removed, and weighed, resulting in an increase in the HFD- group (Table 1), Whereas the weight of WAT was significantly ($p<0.05$) reduced in HFD animals treated with 400 mg/kg. H&E staining (Fig. 1) showed HFD-induced cell enlargement and lipid accumulation, whereas XOC extract indicated a reduced WAT adipocyte size (Fig. 1A). While Fig. 1B displayed the effect of extracts on the liver.

Visceral obesity is the leading risk factor for inadequate accumulation of synergy as triglycerides in WAT adipocytes, and this fat storage is associated with the development of obesity-related disorders, including metabolic syndrome, hypertension, hyperlipidemia, and type 2 diabetes (17). XOC extract reduces the size of adipocytes under HFD-fed treatment, enhances glucose intolerance due to the promotion of adipocyte differentiation, and increases the content of small adipocytes in WAT, like the activity of thiazolidinediones.

Obesity generally is associated with lipid accumulation in the liver leading to the formation of a fatty liver (18). The weight of the liver was significantly ($p<0.05$) increased in HFD. Therefore, the supplementation of HFD + XOC (200, 300 and 400 mg/kg) and HFD + Ph displayed a reduction in

liver weight when compared with HFD mice. However, kidney and spleen weights were not modified during the experiment. The finding indicated that XOC exhibited dose-response relationship.

We carried out a histological assay to evaluate the effect of XOC on the fat accumulation in the liver of HFD mice, hematoxylin-eosin (HE) staining of liver tissue of the HFD group showed injury to hepatocytes with significantly large-sized lipid droplets. Nevertheless, the size of lipid droplets exhibits a reduction in the liver tissue of the groups treated with the extracts. Our results displayed that supplementation of XOC ameliorates hepatic steatosis in HFD-fed mice. Due to its anti-adipogenic effect, XOC extract treatment to HFD mice groups can reduce lipid absorption.

Fig 1. Representative H&E staining of (A) white adipose tissue subcutaneous and visceral (WAT); (B) in the liver of mice groups fed with ND, HFD, or HFD+ XOC (400 mg/kg) for 5 weeks.

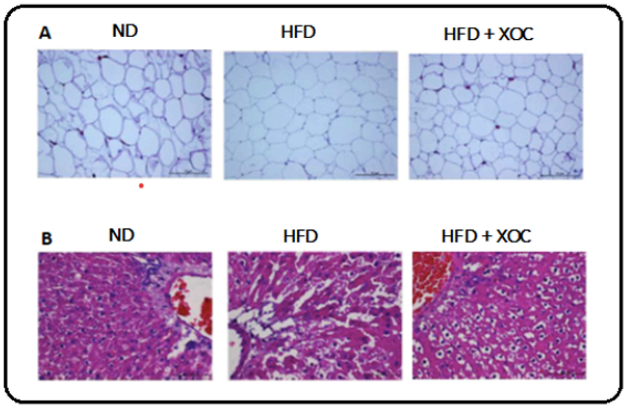

Table 1. Effect of peel of *O. joconostle* Web hydroethanolic extract (XOCO) on various parameters in mice fed high-fat diet for 5 weeks

Parameters	ND	HFD	200mg/kg HFD + XOC	300mg/kg HFD + XOC	400mg/kg HFD + XOC	30mg/kg HFD + Ph
Body weight gain (g/day)	0.089 ± 0.008^{b}	0.461 ± 0.008^{a}	0.312 0.006^{ab}	0.287± 0.005 ^{ab}	0.220± 0.009 ^{ab}	0.285± 0.009^{ab}
Food intake rate (g/day/ mice)	2.7 ± 0.62^{b}	4.0± 0.74^{a}	3.5± 0.61^{a}	3.0± 0.91^{a}	2.6± 0.37^{a}	2.5± 0.68^{a}
Liver weight (g)	1.5± 0.09^{a}	2.0± 0.06^{b}	1.7± 0.08^{a}	1.5± 0.07^{a}	1.4± 0.05^{a}	1.4± 0.03^{a}
Kidney weight (g)	0.4± 0.007	0.4± 0.009	0.4± 0.002	0.4± 0.009	0.4± 0.008	0.4± 0.007
Spleen weight (g)	0.20± 0.002	0.20± 0.007	0.20± 0.008	0.20± 0.006	0.20± 0.001	0.20± 0.004
Abdominal subcutaneous fat (g)	0.26± 0.043^{b}	3.1± 0.081^{a}	2.1 ± 0.07 ^{ab}	1.5± 0.08 ^{ab}	0.93 ± 0.04^{ac}	0.9± 0.01 ^{ac}
Epididymal adipose tissue (g)	0.51± 0.015^{b}	2.77± 0.08^{b}	22± 0.026^{c}	1.6± 0.029^{c}	0.99± 0.039^{c}	1.5± 0.038^{c}
Intestinal adipose tissue (g)	0.43± 0.003^{b}	1.6± 0.007^{a}	1.0± 0.009^{ab}	0.95± 0.006^{ab}	0.80 ± 0.003^{c}	0.89± 0.002^{c}
Glucose (mmol/L)	5.3±0.6^{a}	11.7±1.5^{b}	8.5±1.7^{ab}	8.0±1.6^{ab}	6.8±0.9^{a}	8.4±2.0^{a}
Leptin (ng/mL)	0.3± 0.007^{a}	1.0± 0.005^{b}	0.8± 0.009^{b}	0.7± 0.009 ^{ab}	0.5± 0.002^{c}	0.5± 0.004^{c}
Adiponectin (ng/mL)	2.8± 0.021 ^{a}	2.0± 0.040^{b}	2.3± 0.039	2.5± 0.030^{a}	2.7± 0.015 ^{ab}	2.4± 0.070
Resistin (ng/mL)	18±1.1^{a}	23±3.0 ^{b}	23±3.0^{ab}	20±31 ^{a}	19±2.4 ^{a}	23±3.0 ^{b}
AST (mg/dl)	84.5±6.1a	151.0±3.3^{b}	110.2±6.0 ab	103.7±5.8 ab	88.3±5.5^{a}	111.7±7.3ab
ALT (mg/dl)	22.9±2.4^{a}	64.1±3.8^{b}	44.5±2.8 ^{ab}	37.6±1.9 ^{ab}	28.9±4.0^{ac}	29.9±2.2^{ac}
HDL-CHO (mg/dl)	33.9±4.0^{a}	22.3±5.5^{b}	24.8±2.7^{ab}	26.8±3.2^{ac}	33±4.5^{ac}	31.1±3.7^{ac}
LDL-CHO (mg/dl)	39.2±1.9^{a}	114.3±2.8^{b}	80.6±2.9^{ab}	75.1±5.3^{ab}	63.5±19 ^{ac}	70.7±3.5^{ab}
T-CHO (mg/dl)	117.5±4.5^{a}	253.6±7.2^{b}	162.0±5.7^{ab}	172.8±7.4^{ab}	125.3±5.3^{ac}	142.6±5.2^{ac}
TG (mg/dl)	95.2±3.3^{a}	198.3±4.9^{b}	153.8±5.1 ^{ab}	112.1±6.0 ^{a}	97.3±5.4^{ac}	101.9±3.6^{ab}
NEFA (mg/dl)	3.5±0.06^{a}	4.9±0.07^{a}	4.0±0.03^{b}	3.9±0.1^{b}	3.6±0.05^{ab}	3.7±0.06

Data are expressed as means ±DS (n = 8). AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL-CHO, high-density lipoprotein-CHO; LDL-CHO, low-density lipoprotein-CHO; TG, triglycerides; NEFA, no esterified fatty acid; HFD, high-fat-diet control group; HFD + XOC(200 mg/kg); HDF +XOC (300 mg/kg); HDF + *XOC* (400 mg/kg); and HDF + phentermine (Ph, 30 mg/kg). Data are expressed as means ±DS (n = 8). $^{a–c}$ Values with different letters are significantly different from each other at $p < 0.05$ as determined by Turkey's multiple-range test.

Conclusion

Findings indicated that XOC extract treatment with this medicinal plant in obese mice reduced organ fat weight, adipose tissue weight, the absolute body weight, serum lipid levels, and glucose levels in the serum and improves lipid accumulation in the liver. The screened an anti-obesity supplement from XOC demonstrated that has promising effect. This investigation suggests that flavonoids content might be of importance related to the therapy of obesity recommend its use as a therapeutic anti-obesity agent.

Credit authorship Contributions Statement

Rosa Martha Pérez Gutiérrez designed the data experiment, , performed the in vivo part, interpreted and the statistical analysis, manuscript revision, performed the experimentation, performed results interpretation and corrected the manuscript , interpreted the results, corrected the manuscript and drafted the work.

Declaration of Competing Interest

The authors declare no conflict of interest.

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