Cucurbita ficifolia Bouché (Cucurbitaceae) modulates inflammatory cytokines and IFN-γ in obese mice

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**Cucurbita ficifolia** Bouché (Cucurbitaceae) modulates inflammatory cytokines and IFN-γ in obese mice

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Abstract

This study aimed to investigate the effect of aqueous extract of *Cucurbita ficifolia* on systemic chronic inflammation in an obesity model induced by monosodium glutamate (MSG) via modulating the expression of adipokines (TNF-α, IL-6, resitin and adiponectin) and immune-regulatory cytokines (IFN-γ and IL-10). *Cucurbita ficifolia* extract was administered daily by gavage to lean and MSG-obese mice for 30 days. At the end of treatment, cytokine mRNA expression in adipose tissue was determined by real-time polymerase chain reaction (PCR), and the protein levels of these cytokines were also quantified by enzyme-linked immunosorbent assay (ELISA). *Cucurbita ficifolia* extract decreased body weight and inflammation in MSG-obese mice by reducing the expression of TNF-α and IL-6; these decreases were parallel to significant reductions in protein levels. The extract also increased protein levels of IL-10 in lean mice and IFN-γ in both lean and MSG-obese mice. In conclusion, *Cucurbita ficifolia* extract modulates systemic chronic inflammation in MSG-obese mice and could to have a beneficial effect on the adaptive immune system in obesity.

**Keywords:** Obesity, Monosodium glutamate, *Cucurbita ficifolia*, inflammatory cytokines, IFN-γ.
Introduction

Obesity is a disease of major importance due to its increasing worldwide prevalence; this pathology leads to adverse metabolic effects from glycemia, triglycerides and cholesterol and it is a risk factor for insulin resistance, type 2 diabetes (T2D), dyslipidemias and hypertension (Maury and Brichard 2010; WHO 2014). Obesity has been associated with systemic and chronic low-grade inflammation, particularly due to excess in white adipose tissue (Milner and Beck 2012), which is manifested with augmented synthesis and secretion of adipokines, such as interleukin-6 (IL-6), tumor necrosis factor type alpha (TNF-α) and resistin, resulting in the development of the insulin resistance by desensitizing insulin receptor or decreasing activation of insulin receptor substrate 1, which contributes to the development of T2D (Senn et al. 2003; Steinberg 2007; Torres-Leal et al. 2010).

In addition, the inflammatory state impacts the immunological system, increasing susceptibility to infections (Milner and Beck 2012). Some studies have suggested that obesity alters T-cell production via an increased number of adipocytes and secreted cytokines within the lymphoid microenvironment of the thymus and bone marrow (Yoshida et al. 2014). In addition, obesity involves alterations in the levels of immune-regulatory cytokines, such as interferon-gamma (IFN-γ), interleukin-2 (IL-2) and interleukin-10 (IL-10) (Dicker et al. 2013), which explains the high susceptibility to infections. The development of new anti-inflammatory agents that ameliorate this type of systemic inflammation could prevent the development of the pathologies associated with obesity.
*Cucurbita ficifolia* Bouché has glucose-lowering effects in diabetic animals and T2D patients (Acosta-Patiño et al. 2001; Alarcon-Aguilar et al. 2002). *Cucurbita ficifolia* has also been reported to exhibit antioxidant and anti-inflammatory effects in diabetic mice (Díaz-Flores et al. 2012; Roman-Ramos et al. 2012) and in 3T3-L1 adipocytes (Fortis-Barrera et al. 2013). However, the effect of *Cucurbita ficifolia* on systemic inflammation in obesity has not yet been investigated.

Given the importance of this type of inflammation in obesity, which contributes to diabetes development and high susceptibility to infections, and considering that *Cucurbita ficifolia* has hypoglycemic effects and antioxidant and anti-inflammatory properties, this research aimed to investigate whether an aqueous extract of *Cucurbita ficifolia* ameliorates systemic chronic inflammation in a model of obesity induced by monosodium glutamate (MSG) administration via modulating the expression of adipokines (TNF-α, IL-6, resistin and adiponectin) and the immune-regulatory cytokines IFN-γ and IL-10.

**Materials and methods**

**Preparation and standardization of the *Cucurbita ficifolia* aqueous extract**

Mature fruits of *Cucurbita ficifolia* were collected in the Municipality of Acolman, State of Mexico. Botanical identity was confirmed by the Medicinal Plant Herbarium of the Mexican Institute of Social Security (Specimen Voucher Num. 11119) in Mexico City. The endocarp of fruit free of seeds was dried at room temperature. This dried material (400 g) was ground using a 2 mm mesh in an electric mill (Thomas Wiley Laboratory Mill Model 4, Ramsey, MN, USA) and macerated in water for 72 h. Every 24 h, the aqueous phase was removed and freeze-dried.
As in previous studies (Fortis-Barrera et al. 2013; Roman-Ramos et al. 2012), this extract was standardized in its D-chiro-inositol (DCI) content by high-performance liquid chromatography (HPLC) in a Waters 2697 separation module, a refractive index detector (Waters; Milford, MA, USA) and a LiChrospher NH₂ column (5 µm, 4 x 250 mm, 100 Å°). The elution system was an isocratic mixture of acetonitrile and methanol (8:2). Aliquots (20 µl) of the DCI standard (Sigma Chemical, Steinheim, Germany) (1, 3 and 6 mg/ml) were injected, resulting in a standard linear curve with $R^2=0.99$. The aqueous extract (2 mg/ml (w/v)) was injected into the same HPLC system. DCI had a retention time of 9.3 min, and its concentration was of 3 mg/g extract.

**Monosodium glutamate-induced obesity mice and treatments**

Neonatal mice (CD-1) were used. Pups were subcutaneously administered 2 mg MSG/g body weight (Sigma Chemical, Steinheim, Germany) on postnatal days 2 and 4 and 4 mg/g on postnatal days 6, 8 and 10. MSG was dissolved in 0.01 ml/g body weight of isotonic saline solution (ISS). Another group of pups was subcutaneously administered 0.01 ml ISS/g body weight. Mice were separated after weaning by sex and weighed weekly; only male mice were used.

Mice had free access to water and food (Harlan Laboratories, Indianapolis, USA) and were maintained at an automatic 12-h light-dark cycle. MSG-obese mice exhibited an obesity phenotype, whereas the mice that received ISS did not. At four months of age, the animals were divided into three groups of lean mice and three of MSG-obese mice and received the following treatments by gavage: ISS (4 ml/kg/day), *Cucurbita ficifolia* extract (200 mg/kg/day dissolved in 4 ml of ISS) and
pioglitazone as positive control (0.64 mg/kg/day dissolved in 4 ml of ISS). All experiments were conducted in accordance with the international rules for the care and use of laboratory animals and in agreement with the Mexican Official Norm (NOM-062-ZOO-1999, revised 2001). The Institutional Committee of the Metropolitan Autonomous University at Mexico City approved this protocol.

**Measurement of body weight, food intake and biochemical parameters**

Body weight and food intake were determined throughout the experiments. At the end of treatment, blood glucose concentrations were quantified according to the hydrogenase method (Accutrend Alpha, ROCHE, Mannheim, Germany), A1C was quantified using a DCA 2000 (Bayer, Elkhart, USA) and plasma triglyceride levels were determined with a Reflotron System (Roche Diagnostics, Mannhein, Germany).

**Quantification of cytokines by enzyme-linked immunosorbent assay (ELISA)**

At the end of treatment, the animals were anesthetized with pentobarbital to obtain blood samples from ocular orbital sinus. Serum was recovered by centrifugation at 419 x g for 10 min at 4°C. Cytokine concentrations were measured by ELISA: IL-6 and IL-10 (Pierce; Rockford, USA), tumor necrosis factor receptor 2 (TNFR2), resistin, adiponectin and IFN-γ (R & D, Minneapolis, USA).

**Quantification of adipokine mRNA expression by real-time reverse transcription-polymerase chain reaction**

The animals were euthanized, and the adipose tissue collected and stored at -70°C in RNAlater (QIAGEN Sciences, Germantown, MD, USA) until isolation; total RNA was isolated by using RNeasy Lipid Tissue Mini kit (QIAGEN) following the
manufacturer’s instructions. cDNA was reverse-transcribed using the ImProm II Reverse Transcription System (Promega, Madison, WI, USA). IL-6, TNF-α, resistin and adiponectin genes were amplified in a LightCycler 2.0 Real-Time PCR detection system (Roche Molecular Biochemicals, Indianapolis, IN, USA) with SYBR Green Master Mix (Roche) and the primers shown in Table 1. Relative changes in mRNA expression were obtained by the 2^−ΔΔCt method; mRNA expression is reported as a ratio over the expression level of the housekeeping gene 36B4.

**Statistical analysis**

Data are expressed as the mean ± S.E.M. Differences between treatments were determined by variance analysis, followed by the *post hoc* Tukey–Kramer test for multiple comparisons. Statistical analyses were performed with NCSS 2007 software. A *p* value < 0.05 was considered statistically significant.

**Results**

**Body weight, food intake and biochemical parameters**

At fourth month of age, the MSG-obese mice exhibited an obese phenotype, with significantly increased body weight compared with lean mice (*T*=0). This difference remained constant at 30 days between lean and MSG-obese mice treated with ISS (Fig. 1B). Treatment with *Cucurbita ficifolia* extract significantly decreased body weight in both lean and obese mice compared with the respective untreated control after 30 days of treatment (Figs. 1A-1B, respectively). Food intake in lean mice did not significantly change with the different treatments (Fig. 1C). However, in obese mice, food intake was significantly lower (25%) compared with lean mice, whereas
extract decreased food intake (30%) compared with control obese-ISS (Fig. 1D).

*Cucurbita ficifolia* extract and pioglitazone treatment did not induce change in blood glucose and A1C levels in lean and MSG-obese mice (Table 2). The extract did not alter triglyceride concentrations, which were 70 mg/dl in all cases (data not shown).

**Effects of *Cucurbita ficifolia* extract on TNF-α expression and TNFR2 levels**

In lean mice, *Cucurbita ficifolia* and pioglitazone decreased TNF-α mRNA expression in adipose tissue (60% and 45%, respectively) compared with lean-ISS control (Fig. 2A). The protein serum levels of TNFR2, which are equivalent to TNF-α levels, did not change with *Cucurbita ficifolia* (Fig. 2B) but increased by approximately 30% with pioglitazone. In contrast, MSG-obese mice exhibited a significant increase in TNF-α mRNA expression (170%) and protein levels (24%) of TNFR2 compared with control lean mice (Figs. 2C and 2D, respectively). In MSG-obese mice, treatment with *Cucurbita ficifolia* extract and pioglitazone reduced TNF-α mRNA expression in adipose tissue (140% and 115%, respectively) (Fig. 2C). In addition, *Cucurbita ficifolia* decreased TNFR2 secretion by 23%, whereas pioglitazone did not exhibit any effect in MSG-obese mice (Fig. 2D).

**Effects of *Cucurbita ficifolia* extract on the IL-6 expression and protein levels**

In lean mice treated with *Cucurbita ficifolia* extract, IL-6 mRNA expression decreased by 40% (Fig. 3A); conversely, protein levels of IL-6 were increased by 44% relative to control (Fig. 3B). Pioglitazone treatment did not alter mRNA expression but increased IL-6 protein by 65% (Figs. 3A-3B, respectively). Moreover, compared with lean mice, IL-6 mRNA expression was increased in MSG-obese mice by 150%, without significant changes in IL-6 protein levels (Figs.
3Ch3D, respectively). In MSG-obese mice, treatment with *Cucurbita ficifolia* extract and pioglitazone decreased IL-6 mRNA expression by approximately 89% and 77%, respectively (Fig. 3C). This decrease in mRNA expression was consistent with protein levels in both treatments (Fig. 3D).

**Effects of *Cucurbita ficifolia* extract on resistin expression and protein levels**

*Cucurbita ficifolia* extract and pioglitazone in lean mice increased resistin mRNA expression (130% and 160%, respectively) and protein levels (30% and 20%, respectively) compared with control lean-ISS mice (Figs. 4A-4B, respectively). In MSG-obese mice, resistin mRNA expression and secretion increased by approximately 80% and 75%, respectively, compared with lean mice (Figs. 4C-4D, respectively). The increase in resistin mRNA expression in MSG-obese mice diminished after treatment with *Cucurbita ficifolia* extract and pioglitazone by approximately 100% with both treatments (Fig. 4C). This decrease in mRNA expression was consistent with a diminished protein level of 40% in only the pioglitazone-treated group (Fig. 4D).

**Effects of *Cucurbita ficifolia* extract on adiponectin expression and protein levels**

In lean mice, *Cucurbita ficifolia* extract and pioglitazone did not affect adiponectin mRNA expression compared with control (Fig. 5A). However, the protein levels of this adipokine were significantly reduced by 30% with these treatments compared with lean mice (Fig. 5B). MSG-obese mice did not exhibit significant changes in adiponectin mRNA expression at the protein level compared with lean mice. Interestingly, *Cucurbita ficifolia* extract treatment decreased adiponectin mRNA
expression in MSG-obese mice but did not affect protein concentrations (Figs. 5C-5D, respectively). Pioglitazone treatment did not induce significant changes compared with MSG-obese mice control but induced a significant increase (25%) compared with lean-ISS (Fig. 5D).

Effects of *Cucurbita ficifolia* extract on IL-10 and IFN-γ protein levels

*Cucurbita ficifolia* extract and pioglitazone administration in lean mice increased IL-10 protein levels by 75% and 240%, respectively (Fig. 6A). IFN-γ expression was increased by 80% with extract administration in lean mice (Fig. 6B). In MSG-obese mice, IL-10 and IFN-γ levels were significantly increased (47% and 40%, respectively) compared with lean mice (Figs. 6C-6D, respectively). *Cucurbita ficifolia* extract significantly increased serum IFN-γ levels (58%) in MSG-obese mice (Fig. 6D). However, the extract and pioglitazone did not induce significant changes in IL-10 expression in MSG-obese mice compared with obese ISS control (Fig. 6C).

Discussion

Obesity is considered an imbalance between intake and expenditure of energy, which leads to increment in adipose tissue (Torres-Leal et al. 2010). The MSG-obese model increased body weight due to white adipose tissue accumulation, and exhibited high levels of inflammatory cytokines. However, no changes in the metabolism of glucose and triglycerides were observed in the MSG-obese model, in contrast to other obese models, such as *ob/ob* mice, *db/db* mice and Zucker rats (Carley and Severson 2005), likely because MSG-obese mice present hypofagia (Roman-Ramos et al. 2011; Scallet and Olney 1986).
In relation to *Cucurbita ficifolia* extract in MSG-obese mice, a decrement in body weight was observed, which may be like a consequence of decreased food intake. An effect of the extract on body weight was also observed in lean mice that did not show changes in food intake. The decrease in body weight observed in MSG-mice by *Cucurbita ficifolia* extract is an interesting result that requires to be explored in other models of obesity with hyperphagia, hyperglycemia, dyslipidemia and hypoadiponectinemia.

The anti-inflammatory effect of *Cucurbita ficifolia* extract was previously reported in streptozocin-induced diabetes mice and *in vitro* 3T3-L1 cells (Fortis-Barrera et al. 2013; Roman-Ramos et al. 2012). Taking into account that obesity is a previous condition to other metabolic alterations that involve an inflammatory state, it may be possible of to find new agents to preventing the consequences of the obesity, by reducing body weight and the inflammatory process. Therefore, in the present study it is considered necessary to determine the anti-inflammatory effect of *Cucurbita ficifolia* on the condition obesity.

Inflammatory cytokines levels were affected differently in lean and MSG-obese mice after the treatment with the aqueous extract of *Cucurbita ficifolia*. The extract administration in lean mice increased IL-6 and resistin, which is consistent with previous results in 3T3-L1 adipocytes (Fortis-Barrera et al. 2013) and diabetic mice (Roman-Ramos et al. 2012). Additional studies are needed to explain the mechanism on the stimulation of these adipokines by this extract.

In obesity, the inflammatory state is characterized by changes in the secretion of inflammatory adipokines (Delporte et al. 2004; Milan et al. 2002). Increased
release of TNF-α and IL-6 in inflammation stimulates both the c-Jun amino-terminal kinase and IκB kinase-β/nuclear factor-κB pathways, resulting in up-regulation of potential mediators of inflammation that can lead to insulin resistance (Kahn et al. 2006; Torres-Leal et al. 2010). In the present study, MSG-induced obesity mice increased inflammatory cytokines but did not alter adiponectin, an anti-inflammatory cytokine.

Adiponectin might be expected decreased in MSG-obese model compared with lean mice. However, adiponectin levels stayed without alterations in the obese model. MSG-obese model is characterized by to have normal levels of adiponectin, as it has been reported (Furuya et al. 2010; Roman-Ramos et al. 2011). Adiponectin participates in the regulation of the carbohydrates and lipids (Tao et al. 2014). Thus, MSG-obese mice did not show decreased adiponectin, contrarily at the observed in other models, and in consequence they do not present alterations in carbohydrates nor lipids. We hypothesize that the normal adiponectin levels prevent alterations in lipid and carbohydrates metabolism, although an increase of inflammatory cytokines was showed in obese mice.

In MSG-obese mice, *Cucurbita ficifolia* extract significantly decreased TNFR2 and IL-6 levels, possibly due to the reduction in body weight. Although *Cucurbita ficifolia* extract in obese mice only decreased mRNA resistin expression, and protein levels tended to diminish, the effect was similar that has been shown on 3T3-L1 adipocytes (Fortis-Barrera et al. 2013). Pioglitazone significantly reduced IL-6 and resistin expression, but increased body weight in obese mice, which it is an undesired consequence of thiazolidinediones due to the activation of
peroxisome proliferator-activated receptor gamma (PPAR-γ) in adipose tissue (Tomaru et al. 2009). These results indicate that the anti-inflammatory effect of *Cucurbita ficifolia* extract is different than that exhibited by thiazolidinediones. In genetically obese animals, inflammatory cytokines such as TNF-α from adipose tissue is higher than lean controls and this may affect the immune system, specifically the lymphoid tissue development and induce programmed cell death (apoptosis) (Lamas et al. 2002). Additionally, the secretion of anti-inflammatory cytokines such as IL-10 is impaired in obesity; in contrast, spleen-derived IL-10 protects against inflammatory responses in white adipose tissue and in the liver of obese mice (Gotoh et al. 2012). This cytokine can regulate type 1 T helper (Th1) cell patterns in the cellular immune response (Foss-Freitas et al. 2007). Th1 cells can secrete cytokines (e.g., IFN-γ, IL-4, etc.) and are key players in adaptive immunity; defects in IFN-γ pathway are associated with susceptibility to disease (Chang et al. 2007). In other studies, IL-10 was found reduced and IFN-γ incremented in obese mice (Han et al. 2014).

Here, MSG-obese exhibited high serum IL-10 levels and reduced IFN-γ levels compared with lean mice. High levels of glucocorticoids have been reported in the MSG-obese model (Moreno et al. 2006), which may be associated to high serum IL-10 levels because glucocorticoids regulate the secretion of this cytokine in T cells (Barnes 2011). The increase in IL-10 levels could modulate IFN-γ secretion in T cells. *Cucurbita ficifolia* extract administration restored IFN-γ levels in MSG-obese mice. These IFN-γ results are consistent with previous reports in which *Cucurbita ficifolia*
extract administration increased IFN-γ levels in diabetic mice and also increased IL-10 levels (Roman-Ramos et al. 2012), similar to the behavior observed here in lean mice. Therefore, the effects of plant extract on immune-regulatory cytokines may be an interesting alternative for preventing or reducing immune alterations in obesity and other pathologies, although additional studies are needed.

The main contribution of this manuscript is the effect of the extract of *Cucurbita ficifolia* on inflammatory cytokines in an obesity condition. This extract may be an alternative for treatment of inflammation due to obesity without increasing body weight. The findings suggest a different mechanism between the extract and thiazolidinediones. Since these drugs have an anti-inflammatory effect but increase body weight (Corzo and Griffin 2013).

In previous studies, DCI has been used as mark to standardize this extract (Fortis-Barrera et al. 2013; Roman-Ramos et al. 2012). However, its participation in this action is yet unclear. A comparative analysis of the actions produced by DCI and the *Cucurbita ficifolia* extract on cytokine secretion and activation of PKB (a protein involved in the insulin pathway) in 3T3-L1 adipocytes showed substantial differences (Fortis-Barrera et al. 2013). In addition, the amounts of inositol used in the present investigation are low for the effects showed by the extract. Therefore, identifying other compounds in the extract that could be responsible for these actions is mandatory.

Several flavonoid antioxidant molecules have been proposed to affect IFN-γ, which could activate the enhancing activity of the nuclear factor of activated T cells (NFAT) and nuclear factor kappa B (NF-κB), which are involved in IFN-γ
transcription (Chang et al. 2007). In addition, cucurbitacin-type molecules could be present in *Cucurbita ficifolia* extract, because they are normally present in the Cucurbitaceae family (Jayaprakasam et al. 2003). Since these compounds exhibit anti-inflammatory effects, they should be explored in further experiments. In conclusion, *Cucurbita ficifolia* extract decreased body weight, accompanied by decreases in mRNA expression and protein levels of TNFR2 and IL-6 in MSG-obese mice. Interestingly, *Cucurbita ficifolia* extract also increased protein levels of IL-10 in lean mice and IFN-γ in both lean and MSG-obese mice. The effect observed on weight loss in this MSG model, suggests that the extract can prevent obesity and its subjacent systemic inflammation. Additional studies are needed to explain the implications of the affectation of IFN-γ in obesity by this extract.

**Acknowledgments**

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**References**


Steinberg, G.R. 2007. Inflammation in obesity is the common link between defects in fatty acid metabolism and insulin resistance. Cell Cycle, 6(8): 888-894. PMID:17438370.


Table 1. Primer sequences used for RT-PCR

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Table 2. Effect of the daily administration for 30 days of *Cucurbita ficifolia* extract on glycaemia and A1C, in lean mice and MSG-obese mice (mean ± S.E.M. *n* = 4).

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Figure 1. Effect of *Cucurbita ficifolia* (*C. ficfolia*) extract on body weight and food intake in lean and obese mice. Body weight (A and B) and food intake (C and D) were determined throughout the experiments (30 day). Values are reported as the mean ± S.E.M. (*n* = 4). §Significant difference compared to the lean-ISS control group; *Significant difference compared to the obese-ISS group (*p* < 0.05, ANOVA).

Figure 2. Effect of *Cucurbita ficifolia* (*C. ficfolia*) extract on mRNA expression and protein levels of TNF-α in lean and obese mice. mRNA expression of TNF-α in adipose tissue was analyzed using RT-PCR in lean (A) and obese mice (C), and the serum levels of TNFR2 were determined by ELISA in lean (B) and obese mice (D) after 30 days of treatment with *C. ficifolia* (200 mg/kg/day). A positive control (pioglitazone, 0.64 mg/kg/day) was included. Values are reported as the mean ± S.E.M. (*n* = 4). §Significant difference compared to the lean-ISS control group; *Significant difference compared to the obese-ISS group (*p* < 0.05, ANOVA).

Figure 3. Effect of *Cucurbita ficifolia* (*C. ficfolia*) extract on mRNA expression and protein levels of IL-6 in lean and obese mice. mRNA expression of IL-6 in adipose tissue was quantified by RT-PCR in lean (A) and obese mice (C), and the protein levels of IL-6 were determined by ELISA in lean (B) and obese mice (D) after 30 days of treatment with *C. ficifolia* (200 mg/kg/day). A positive control (pioglitazone, 0.64 mg/kg/day) was included. Values are reported as the mean ± S.E.M. (*n* = 4). §Significant difference compared to the lean-ISS control group; *Significant difference compared to the obese-ISS group (*p* < 0.05, ANOVA).

Figure 4. Effect of *Cucurbita ficifolia* (*C. ficfolia*) extract on the mRNA expression and protein levels of resistin in lean and obese mice. mRNA expression of resistin in adipose tissue was analyzed using RT-PCR in lean (A) and obese mice (C), and the serum levels of resistin were determined by ELISA in lean (B) and obese mice (D) after 30 days of treatment with *C. ficifolia* (200 mg/kg/day). A positive control (pioglitazone, 0.64 mg/kg/day) was included. Values are reported as the mean ± S.E.M. (*n* = 4). §Significant difference compared to the lean/ISS control group; *Significant difference compared to the obese-ISS group (*p* < 0.05, ANOVA).
Figure 5. Effect of *Cucurbita ficifolia* (*C. ficfolia*) extract on mRNA expression and protein levels of adiponectin in lean and obese mice. mRNA expression of adiponectin in adipose tissue was quantified by RT-PCR in lean (A) and obese mice (C), and the protein levels of adiponectin were determined by ELISA in lean (B) and obese mice (D) after 30 days of treatment with *C. ficifolia* (200 mg/kg/day). A positive control (pioglitazone, 0.64 mg/kg/day) was included. Values are reported as the mean ± S.E.M. (*n* = 4). §Significant difference compared to the lean-ISS control group; *Significant difference compared to the obese-ISS group (*p* < 0.05, ANOVA).

Figure 6. *Cucurbita ficifolia* (*C. ficfolia*) extract on serum levels of immunoregulatory cytokines, IL-10 and IFN-γ. Serum levels of IL-10 (A) and IFN-γ (B) were determined by ELISA in lean after 30 days of treatment with *C. ficifolia* (200 mg/kg/day). Also, in obese mice were measured IL-10 (C) and IFN-γ (D). A positive control (pioglitazone, 0.64 mg/kg/day) was included. Values are reported as the mean ± S.E.M. (*n* = 4). §Significant difference compared to the lean-ISS control group; *Indicates statistically significant difference compared to the obese-ISS group (*p* < 0.05, ANOVA).
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