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Consumption of *Ocimum sanctum* L. and *Citrus paradisi* infusion modulate lipid metabolism and insulin resistance in obese rats

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**ABSTRACT**

High saturated fat and fructose diet leads to metabolic disorders through dysregulation of genes involved in lipid metabolism. Consumption of plant infusions reduces these obesity alterations, but the precise mechanism remains unclear. In this study, we investigated the effect and the possible mechanism of *Ocimum sanctum* L. (OS) and *Citrus paradisi* (CP) infusions in diet-induced obese rats. CP and OS infusions suppressed hepatic tissue fat accumulation, and significantly down-regulated the mRNA levels of two hepatic lipogenesis genes: sterol regulatory element binding protein 1c (SREBP1c) and fatty acid synthase (FAS) compared with obese control. Treatment with these infusions up-regulated the hepatic expression of mRNA related with mitochondrial fatty acid uptake: peroxisome proliferator activated receptor alpha (PPARα) and the expression of carnitine palmitoyl-transferase 1a (CPT1a). Both infusions improved insulin resistance, OS showing the major effect. Consumption of these infusions reduces the damage caused by free radicals, protecting hepatic lipid and proteins. Additionally, plant infusions increase activity of hepatic enzymes: glutathione S-transferase (GST), glutathione peroxidase (GPX), and catalase (CAT). Our results suggest that the effects of CP and OS infusions on lipid metabolism are related to the down-regulation of genes involved in lipogenesis, particularly for OS, and with the increase in lipid β-oxidation, especially for CP infusion. In conclusion, the consumption of these plant infusions is a feasible adjuvant therapy for metabolic changes induced by obesity.

**Keywords:** *Hibiscus sabdariffa*; *Ocimum sanctum* L.; *Citrus paradisi*; Obesity, lipid metabolism, insulin resistance.

**Abbreviation list:** *Hibiscus sabdariffa* (HS), *Citrus paradisi* (CP), *Ocimum sanctum* L. (OS) High-saturated-fat and fructose diet (HFFD), Triglycerides (TG), Free fatty acids (FFA), hepatic insulin resistance (HIR).
1. Introduction

Obesity is the most prevalent metabolic disease in the western world which is related to high consumption of calories from high saturated fat food and caloric beverages, especially those sweetened with fructose.\(^1\) Excess of dietary carbohydrates are transformed in triglycerides (TG) in the liver and subsequently stored in white adipose tissue. A flaw in free fatty acid (FFA) oxidation or TG exportation leads to an increase in the lipid stored in hepatocytes in form of TG condition that eventually produce steatosis.\(^2\)

Previous studies demonstrated that the development of steatosis is associated with an increase of lipogenesis, mediated by an increase in the activity of the transcription factor: sterol regulatory element–binding protein-1c (SREBP-1c).\(^3\) SREBP-1c regulates genes required for lipogenesis such as fatty acid synthase (FAS).\(^4\) Another factor associated to steatosis is the decrease oxidation of the lipids into the mitochondria. β-oxidation is regulated mainly by the transcription factor: peroxisome proliferator activated receptor alpha (PPARα). PPARα regulates the expression of genes that participate in FFA oxidation (included CPT1a).\(^5\) Down-regulation of PPARα and CPT1a could be related to a development state of steatosis.

Several studies have documented a strong relationship between steatosis and hepatic insulin resistance (HIR), with an overproduction of glucose despite the presence of high levels of circulating insulin.\(^6\) Furthermore, evidence \textit{in vitro} indicates that steatosis and HIR is accompanied by numerous adverse effects on mitochondrial function, including the uncoupling of oxidative phosphorylation,\(^7\) thus with generation of reactive oxygen species (ROS), including superoxide.\(^8\)
Additionally, excess of lipids also increase HIR through decreased endogenous antioxidant defenses such as hepatic glutathione (GSH) levels and catalase (CAT) activity.\textsuperscript{9,10} Several studies on obesity treatment and its complications have focused on the potential that plant infusions exert on lipid metabolism. Previous reports showed that *Hibiscus sabdariffa* (HS) aqueous extract decreases body weight gain and liver steatosis in diet-induced obese mice; this latter through down-regulated SREBP-1c.\textsuperscript{11} Treatments with *Ocimum sanctum* L. (OS) leaf extract showed a decreased hyperlipidemia and improved oxidative state in liver and heart in high-cholesterol diet fed rats.\textsuperscript{12} Similarly, treatment with *Citrus paradisi* (CP) significantly decreases total cholesterol and low-density lipoprotein.\textsuperscript{13} However to our knowledge, the mechanism of the effects on lipid metabolism of these plants has been not reported.

In the present study, we investigated the effect of OS and CP infusions on lipid metabolism and insulin resistance, using obese rats induced with high-saturated fat and fructose diet (HFFD).

2. Materials and methods

2.1 Preparation of plant infusions

We used three different plant materials: *Citrus paradisi* (CP), *O. sanctum* L. (OS), and *Hibiscus sabdariffa* (HS); this latter was used for comparative purposes. CP and HS were obtained at the locality Apatzingan (Michoacán, Mexico) and Santo Domingo (Guerrero, Mexico) respectively in properly managed fields. OS was collected from a greenhouse (Querétaro, Mexico). A representative sample from the production of the 2011 season was collected. CP (previously sliced in transverse...
sections) and OS were dried in a convection oven at 50 ºC for 24 h. HS flowers were
sun dried at ambient temperature according to the instructions from the producers.
Dried materials were ground (1.13 mm), afterwards stored in the darkness for later
analysis. Infusions (1 %) were prepared by adding 200 mL of freshly boiled water to
2 g of dried ground sample, then let stand for 10 minutes and strained using a
commercial paper filter.

2.2 Animals and experimental design
Male Sprague-Dawley rats (Rismart SA de CV, Mexico City, Mexico), eight weeks of
age, were housed in a light and temperature-controlled room (12-12 h light-dark
cycle; 24 ± 1 ºC). Experiments on animals were performed in accordance with the
Animal Care and Use protocol of the University of Queretaro, as recommended by
NIH (Publications No. 80-23).
Rats were randomly divided into groups of seven animals. Healthy control group
was fed with a base diet (proteins 22 %, lipids 5 % [1 % saturated fat], and
carbohydrates 45 %). Obese control, HS, OS, and CP groups were fed with a high
fructose and high saturated fat diet (HFFD) (proteins 14 %, lipids 40 % [38 %
saturated fat], and carbohydrates 51 % [17 % fructose]) for nine months. Starting the
sixth month, instead of water, infusion treatments (1 %) were administered to HS,
OS, and CP groups. Measurement of body weight was performed weekly; food and
infusion intake were performed daily.

2.3 Measurements and blood sampling
After nine months, rats were sacrificed. Liver and adipose tissue were removed,
weighed, and immediately frozen in liquid nitrogen, stored at 80 ºC until analysis. A
fraction of omental adipose tissue and liver from each sample was immersed in 10%
formalin solution.

Blood was withdrawn via cardiac puncture; serum was separated and immediately
frozen at 80 °C until analysis. Glucose, serum triglyceride (TG), total cholesterol
(TC), and high-density lipoprotein cholesterol (HDL-c) concentrations were
measured using commercial assay kits (Randox Laboratories Ltd.). Serum insulin
levels were measured using a rat insulin ELISA kit (Millipore, USA). Afterwards, the
insulin resistance index was calculated with the Homeostasis Model Assessment
(HOMA) using the values from insulin and glucose under fasting conditions:

\[
\text{HOMA} = \text{fasting insulin (µU mL}^{-1}) \times \text{[fasting glucose (mmol L}^{-1}) 22.5}^{-1}\]

2.4 Measurement of triglyceride concentration in liver and adipose tissue

Lipids from omental adipose tissue and liver were extracted in a process previously
described.\(^{14}\) The extract was washed with 0.2 volumes of saline solution (NaCl
0.9%) and centrifuged at 4,000 x g for 10 min. The lipid phase was then recovered,
and triglyceride content was determined using an enzymatic method (Randox
Laboratories Ltd.).

2.5 Histology and cell-size measurement

Paraffin embedded fraction of liver and omental adipose tissue from each sample
was used for steatosis and adipocyte size evaluation. Samples were observed and
photographed (100X for adipose tissue and liver, 400X only for liver) in hematoxylin-
eosin–stained tissue sections (5-µm). Adipocyte mean volumes were determined by
computerized image analysis (KS400 software, Carl Zeiss, Oberkochen, Germany).
Cells were examined by selecting five sections for each fat sample; systematic random sampling was used to select ten fields for each section. Cell volume was calculated as follows, volume = $4(3\pi r^3)^{-1}$ (where $r$ is radius); results were expressed in picoliters.$^{15}$

### 2.6 mRNA isolation and cDNA synthesis

Total RNA from liver tissue was obtained using a Total RNA Isolation System (Promega, Madison, MI). Its concentration and purity were measured with a NanoDrop ND-1000 instrument (Thermo Scientific, Wilmington, DE, USA), and the integrity was verified after electrophoresis in a 1% agarose gel stained with SYBR® Gold Nucleic Acid Gel Stain (Invitrogen) under UV-light. cDNA synthesis was performed using 2 µg of total RNA and 1 µL of oligo dT (15 bases, 0.25 µM) plus 8 µL of M-MLV 5X reaction buffer, 1 µL 10 mM dNTP mix (Invitrogen), 1 µL (200 U) of M-MLV RT (Promega, Madison, WI), and RNAse-free water, reaching a final volume of 20 µl and samples were incubated at 37 °C for one h, then stopped by heating to 55 °C for 15 min.

### 2.7 Real-time PCR

The cDNA samples were diluted 1:10 and used as templates for PCR in a LightCycler1 (Roche Diagnostics, Indianapolis, IN, USA). qPCR amplification was performed with the lightcycler Fast Start DNA master SYBR Green I from Roche using 3 µL of the diluted cDNA, 0.5 µM of the corresponding oligonucleotides (Table 1). PCR conditions were as follows: 95 °C 1’, 40 cycles: 95 °C 10” 68 °C 10” and 72 °C 12”. PCR product identity was confirmed by sequencing in an ABI prism 310 from Applied Biosystems, big dye v 3.1. The results were evaluated with the $2^{-\Delta\Delta CT}$
method\textsuperscript{16} using the geometric mean of SOD and CYCA as reference genes. mRNA expression in treated animals is reported as fold difference to obese control.

2.8 Hepatic markers of oxidative stress

Liver was homogenized in 50 mM phosphate buffer (pH 7), containing 0.5 mM EDTA and 0.5 % Triton, using a tissue homogenizer (Kinematica, Switzerland) and centrifuged at 8,000 \(g\) for 15 min at 4 \(^\circ\)C. Homogenized liver was then centrifuged at 8,000 \(g\) for 15 min at 4 \(^\circ\)C. The cytosolic fractions were stored at -70 \(^\circ\)C until analysis. Catalase (CAT), Glutathione peroxidase (GPx), and Glutathione S-transferase (GST) activity was assayed.\textsuperscript{17-19} Results were expressed as \(\mu\text{mol min}^{-1}\ \text{mg protein}^{-1}\) respectively. Extinction coefficient of 6.22 \(\text{mM}^{-1}\text{cm}^{-1}\) was used to calculate GPx. Protein concentration in the cytosolic fractions was determined by the bicinchoninic acid protein assay (Pierce Inc., Rockford, IL), using bovine serum albumin as a standard. Lipid peroxidation was determined by the thiobarbituric acid reaction (TBARS) with malondialdehyde (MDA), a product formed due to the peroxidation of lipids\textsuperscript{20} and expressed as nmol mg protein\(^{-1}\). Oxidative protein damage was measured by protein carbonyl levels (C=O) and determined as previously described.\textsuperscript{21} Carbonyl content was calculated from the maximum absorbance using a molar absorption coefficient of 22,000 \(\text{M}^{-1}\text{cm}^{-1}\). Protein content was determined by using bovine serum albumin as the standard.\textsuperscript{22}

2.9 Statistical analysis

Data were expressed as mean values ± standard error (SE). Statistical significance was determined by one-way variance analysis (ANOVA) (\(P < 0.05\)) followed by the Tukey’s test multiple comparisons. Kruskal-Wallis one way analysis of variance on
ranks and multiple comparison procedures by Dunn's Method was performed for enzymatic activity. All statistical analyses were performed using JMP 5.0.1 software.

3. Results

3.1 Plant infusions (HS, OS, and CP) and its effect on body weight, lipid accumulation in liver and omental adipose tissue.

After nine months following their feeding pattern, obese control weighed around 25 % more than animals fed with normal diet (data not shown). The same trend was observed in the liver and adipose tissue weight (Table 2). The amount of adipose tissue increased more than 3 times for the obese control, and its content of TG was almost 3 times higher compared to healthy control (Table 2). The adipocyte volume augmented around 3.5 times respected to that of rats fed with a normal diet (Figure 1).

HFFD feeding in obese control led to significant increase in liver weight (around 40 %). In addition, hematoxylin-eosin-staining of liver section showed that HFFD induced severe steatosis (85-95 %) characterized by the presence of large and medium lipid droplets, as well as altered hepatocytes and sinusoids (Figure 2). These results were in agreement with liver triglycerides content, which was 2.7 fold higher compared to the healthy control (Table 2).

Animals fed with HFFD and treated with HS infusion decreased body weight (10 %), whereas the other two groups treated with CP and OS did not change their weight (data not shown) compared to obese control animals. No significant statistical difference of food (range of food intake was 20-23 g rat\(^{-1}\) day\(^{-1}\)) and infusion intake [Obese control (water intake): 32.1 ± 2.4 mL rat\(^{-1}\) day\(^{-1}\); HS treated group: 28.9 ± 2.7 mL rat\(^{-1}\) day\(^{-1}\)].
mL rat⁻¹day⁻¹; OS treated group: 33.6 ± 2.4 mL rat⁻¹day⁻¹; CP treated group: 32.4 ±
2.2 mL rat⁻¹day⁻¹] were observed between treated groups and obese control.

In agreement with the effect of infusions on body weight, only HS was able to
decrease the weight of omental adipose tissue (29 %), while the other two infusion
treatments did not lead to any difference compared with the obese control group
(Table 2).

In contrast, rats under HFFD, and treated with plant infusions exhibited decreased
adipocyte volume (Figure 1). Group treated with HS infusion showed adipose cell
reduction (43 %), and their adipocytes showed minor volume (1.8 fold) compared to
adipocytes from obese control rats. OS and CP treated groups also exhibited a
reduction in adipocyte size and volume, but showed intermediate values between
the obese control and the HS treated group (Figure 1). These results are in
agreement with levels of adipose tissue TG, which were reduced in 46 %, 16 % and
38 % for HS, CP and OS respectively.

Treatment with infusions (HS and OS) decreased the weight of liver up to 21 %;
however, these values were not statistically significant (Table 2). Nonetheless, rats
treated with HS had a liver with preserved architecture and lower steatosis (30-40
%). OS showed intermediate values (50-60 %) between HS and obese control. CP
treated group showed a less reduction of steatosis (20-30 %) compared with the
other treated group (Figure 2).

The positive effect of plant infusions on liver steatosis was associated with a
reduction of hepatic TG content, which was decreased considerably compared with
their obese counterparts, up to 59 % and 57 % for HS and OS respectively. CP
decreased only 31 % (Table 2).
3.2 Plant infusions (HS, OS, and CP) regulate mRNA hepatic gene levels involved in lipid metabolism

We studied the expression of two lipogenic genes (SREBP-1c and FAS), which were selected according to their metabolic functions involved in the biosynthesis of fatty acids. There was an increase in SREBP-1c and FAS gene expression in HFFD-fed animals, nearly 10 times compared with that of the healthy control group (Figure 3). These results are in agreement with the increment of steatosis and hepatic TG content. Treatment with the three infusions reduced the expression of these two genes, being HS the most effective followed by OS and finally CP (Figure 3).

Two genes related with fatty acid β-oxidation (PPARα and CPT1a) were also measured. Animals fed with HFFD exhibited a relative expression of the PPARα gene five times lower than the healthy control (Figure 4). Similarly, the expression of the CPT1a gene was reduced 13 times in liver (Figure 4). The best recovery of PPARα expression was observed in rats treated with HS (4 fold) followed by animals treated with OS (3 fold) infusions. Although, CP infusion treatment showed only a slight increase in the expression of PPARα, this infusion consumption recovered the expression of CPT1a by a factor of six in liver (Figure 4).

3.3 Effect of Infusion treatments (HS, OS, and CP) on hyperlipidemia and hyperglycemia.

Obese control presented hyperlipidemia, hyperglycemia, and insulin resistance at the end of the experimental period. We observed elevated quantities of TG in serum from HFFD fed rats, 1.6 fold compared to healthy control (Table 3). There was a hypolipidemic effect in rats receiving infusions in contrast to the obese control. We observed a reduction in serum TG: 34 % for HS, 48 % for CP, and 27 % for OS.
infusions (Table 3). Serum cholesterol concentration was not altered with HFFD; however, HFFD decreased HDL-c levels in obese rats. All plant infusion treatments increased HDL-c concentration, being similar to the healthy group (Table 3). HFFD-fed animals presented values of circulating glucose, which are considered pre-diabetic (Table 3). Additionally, obese control displayed a 1.7-fold increase in insulin over the non-obese control group. Therefore, the HOMA value increased almost 4 times (Table 3). Plant infusion consumption improved diabetic parameters in obese treated rats. A significant decrease in circulating levels of glucose and insulin was observed for the three treated groups; consequently, HOMA values also declined. Results for HS and OS were close to those of healthy control animals (Table 3).

3.4 Effect of Infusion treatments (HS, OS, and CP) improves hepatic oxidative markers.

As expected, HFFD feeding decreased activity in CAT, GPx, and GST enzymes, increased hepatic lipid peroxidation (expressed as TBARS), and hepatic proteins oxidation (expressed as C=O) in comparison with healthy rats (Table 4). HS and OS infusion treatment on obese rats reestablished the enzymatic antioxidant system to normal levels (Table 4). These two infusions displayed enzymatic activity (CAT, GPx, and GST) similar to the non-obese animals and clearly different from the obese group, except for GST in rats treated with HS. CP infusion produced slight but significant increments in GPx activity compared with that of the obese control rats (Table 4). In addition, we demonstrated that the consumption of infusions reduced the levels of MDA, a marker of lipid peroxidation (approximately 84 %), particularly those treated with HS and OS infusion (Table 4). Treatment with infusions
diminished hepatic protein oxidation compared with obese control group (up to 36 %).

4. Discussion

Elevated consumption of caloric diets resulted in metabolic alterations, leading to physiological changes in organs such as adipose tissues and liver. In this study, we examined the effects of CP and OS infusion on HFFD fed obese rats. We included HS infusion as an infusion control, due to its reported anti-obesity potential and elucidated mechanism of action.\textsuperscript{23,24}

Results showed that treatment with these plant infusions improved alterations related with obesity. Most of the reported health benefits of these plant infusions are associated with their bioactive compounds such as polyphenols. Among the compounds reported for these plant materials are: cyanidin-3-sambubioside, protocateuic, coumaric and chlorogenic acid, for HS, naringin and narirutin for CP, and kaempferon and rosmarinic acid for OS.\textsuperscript{25-27} Rats treated with infusions consumed some of these polyphenols, for example: HS treated group 4.6 µg rat\(^{-1}\) day\(^{-1}\) of coumaric acid, OS 4.1 µg rat\(^{-1}\) day\(^{-1}\) of rosmarinic acid, and CP 2.4 µg rat\(^{-1}\) day\(^{-1}\) of naringin (Data obtained by HPLC analysis, Anexo 1). In a previous report, these compounds have demonstrated anti-obesity, antioxidant, and anti-inflammatory effects.\textsuperscript{28}

We analyzed the omental adipose tissue since previous reports have demonstrated that this tissue differs metabolically from other fat cells. For example, omental fat cell enlargement in obesity increases the production of cytokines more than other fat tissues. These cytokines are associated with the development of insulin
In our study, consumption of infusions (OS and CP) decreased adipocyte volume and TG in adipose tissue. Plant infusions also reduce hyperlipidemia and lowered lipid accumulation in liver, thus the presence and progression of steatosis. In order to demonstrate whether this effect is related to the modulation of lipid metabolism by infusions, we evaluated the expression of genes involved in synthesis and oxidation of FFA in the liver. We observed a down-regulation of SREBP-1c and FAS because of the consumption of HS infusion. In agreement with our results, previous studies reported that an HS aqueous extract exerted a hypolipidemic effect, reduced fat liver accumulation, and decreased the lipid content of hepatocyte through down regulated of SREBP-1c.\(^{30}\)

Similarly to HS, we have shown that treatment with OS and CP infusion decreased the expression of both genes compared with the obese control, being more important for group treated with OS. This down-regulation suggests that the attenuation observed in liver steatosis by treated groups is associated with minor fatty acid synthesis.

Reduction of hepatic FFA levels induced by infusions could be related with an increased mitochondrial fatty acid uptake and oxidation through the up-regulation of PPAR\(\alpha\) and CPT1a expression.\(^{31}\) Previous studies report that HS extract showed an enhancement of lipid metabolism through up regulation of the PPAR\(\alpha\) expression in type 2 diabetic rat model.\(^{24}\) Similarly to HS, we demonstrated that OS and CP infusions induced the relative expression of PPAR\(\alpha\) compared with obese control.

On the other hand, rats treated with CP infusions showed a main up regulation of CPT1a (1.5 fold) compared to OS. This could be because the transcriptional
regulation of CPT1a involves several PPARα-independent pathways such as peroximal proliferator activated receptor gamma cofactor 1 (PGC1). Previous studies report that up-regulation of CPT1a reduces hepatic steatosis and insulin resistance in genetically obese db/db mice. CPT1a controlled the entry of long chain fatty acids through the mitochondrial outer membrane by binding them to carnitine in order to carry out β-oxidation. Therefore, our results suggest that proportional decreased of hepatic steatosis observed in CP group, could be associated to mitochondrial fatty acid uptake. However, this mechanism was lower compared with FFA synthesis.

Treatment with CP infusions also improved the concentration on serum TG. This effect could be related to the decreased absorption of dietary lipids. We found that consumption of CP infusion increased the TG in feces of HFFD fed rats (1.4-fold that obese control) (data not published). In agreement with our results, other authors reported that hypolipidemic action in plant materials is probably due to the ability of bioactive compounds to modify absorption and intracellular transport of lipids. Steatosis seems to be one of the first steps in the development of insulin resistance, and then of diabetes type 2. Therefore, we evaluated the hypoglycemic effect of the infusions. All treatment with plant infusions significantly decreased circulating levels of glucose and insulin; consequently, the HOMA values declined more than 50%, OS showed quantities similar to healthy control. These data are in agreement with several studies performed with ethanolic, methanolic, and non-infusion aqueous extracts of different parts of these plant materials. One possible mechanism for the hypoglycemic action of infusions may be attributed to an increased utilization of glucose by the liver for glycogen synthesis. As well as a reduction of
gluconeogenesis, decreasing sugar delivery into the blood stream.\textsuperscript{36} However, there is insufficient research providing evidence for these effects exerted by such infusions (OS and CP).

Improvement of insulin resistance was accompanied by a decrease in steatosis and hepatic oxidative stress markers. \textit{In vitro} evidence indicates that elevated lipid levels induce the synthesis of ROS and impair endogenous antioxidant defenses.\textsuperscript{8} High levels of ROS and depleted endogenous antioxidant system lead to the activation of multiple serine/threonine kinases signaling cascades. These activated kinases can act on a number of potential targets in the insulin signaling pathway, including the insulin receptor and inducing insulin resistance.\textsuperscript{37} This latter effect was observed in HFFD through a decreased activity in CAT, GPX, and GST enzymes, and an increased hepatic lipid and protein oxidation.

However, rats treated with plant infusions restored hepatic endogenous antioxidant system, mainly OS. These results indicate that consumption of infusions could either increase the biosynthesis of antioxidant enzymes or reduce their degradation. The improvement in activity of antioxidant enzymes by infusions can be related to some compounds of plant infusions quantified in this work (Anexo 1), such as rutin and coumaric acid. It has been reported that the treatment with these compounds enhanced the levels of GPx and GST in the hepatic tissue of rats with HFD-induced obesity.\textsuperscript{28}

We also demonstrated that treatment with infusions reduced MDA and protein oxidation due to its antioxidant activity. In agreement with our results, several studies report that different extracts of these plants exhibit antioxidant activity. For instance, treatment with aqueous HS extract reduces lipid peroxidation attributed to
the improvement in the cellular antioxidant system in high fat diet-induced obese mice. Furthermore, OS leaf extract suppressed the high levels of serum lipid profile and hepatic lipids through the reduction in TBARS and raised the low activities of GPx and CAT in rats fed with high-cholesterol (HC) diet. Therefore, OS and CP infusions are an interesting target to improve the metabolic alterations studied in this work.

5. Conclusion

Consumption of OS and CP infusions decreased liver fat accumulation through the down-regulation of gene involved in lipogenesis (SREBP-1c and FAS) and lipid oxidation (PPARα and CPT1a), being better for OS. These regulations are associated with an improvement in insulin resistance and hepatic oxidative stress, thus suggesting that plant infusion (OS and CP) consumption is a viable alternative treatment for these metabolic alterations in obesity induced by obesogenic diets.

Acknowledgments

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6. References


**Table 1.** Primer pairs used in the real-time quantitative PCR reactions.

<table>
<thead>
<tr>
<th>Gene</th>
<th>NCBI ID</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>SREBP-1c&lt;sup&gt;c&lt;/sup&gt;</td>
<td>AF286470</td>
<td>GCCCACAATGCCATTGAGA</td>
<td>CAGGTCTTGAGCTCCACAATC</td>
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<tr>
<td>FAS</td>
<td>NM_017332</td>
<td>GGACATGGTCACAGACGATGAC</td>
<td>GTGAACTTGGACAGATCTTCA</td>
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<td>PPARα</td>
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<td>CPT1</td>
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<tr>
<td>SOD</td>
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<td>CYCA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>XM_345810</td>
<td>AGCAGCTGGGAGAAAGATT</td>
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Table 2. Relative organ weight and lipid concentration of obese rats treated with infusions. Values are expressed as mean ± SE (n = 7).

<table>
<thead>
<tr>
<th>Organ Weight</th>
<th>Healthy Control</th>
<th>Obese Control</th>
<th>HS</th>
<th>CP</th>
<th>OS</th>
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</thead>
<tbody>
<tr>
<td>Liver (g)</td>
<td>9.37 ± 0.6c</td>
<td>15.24 ± 0.50a</td>
<td>12.08 ± 1.1b</td>
<td>14.40 ± 0.60a</td>
<td>12.63 ± 0.90b</td>
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<tr>
<td>Abdominal Adipose Tissue (Omental) (g)</td>
<td>4.44 ± 0.3b</td>
<td>17.72 ± 1.0a</td>
<td>12.44±3.2a</td>
<td>16.59±1.0a</td>
<td>17.82±1,0a</td>
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</table>

Lipids in Organs

<table>
<thead>
<tr>
<th>Hepatic Triglycerides (mg g⁻¹)</th>
<th>26.8 ± 4.10b</th>
<th>72.7 ± 3.90a</th>
<th>29.6 ± 3.10b</th>
<th>50.2 ± 1.10c</th>
<th>31.4 ± 5.30b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omental Adipose Tissue Triglycerides (mg g⁻¹)</td>
<td>82.7 ± 5.10d</td>
<td>239.7 ± 6.30a</td>
<td>129.6 ± 16.90cd</td>
<td>201.5 ± 13.40b</td>
<td>147.2 ± 5.00c</td>
</tr>
</tbody>
</table>

Values in a row followed by different letters are significantly different (p < 0.05) by Tukey’s test. *Hibiscus sabdariffa* (HS), *Citrus paradisi* (CP), *Ocimum sanctum* L. (OS).
Table 3. Serum metabolites and Homeostasis Model Assessment (HOMA) of obese rats treated with infusions. Values are expressed as mean ± SE (n = 7).

<table>
<thead>
<tr>
<th></th>
<th>Healthy control</th>
<th>Obese control</th>
<th>HS</th>
<th>CP</th>
<th>OS</th>
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<tbody>
<tr>
<td><strong>Total Cholesterol (mg dL⁻¹)</strong></td>
<td>94.3 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.0 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102.3 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>109.0 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.2 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>High Density Lipoprotein (mg dL⁻¹)</strong></td>
<td>52.30 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.34 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.1 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.3 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.0 ± 5.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Triglycerides (mg dL⁻¹)</strong></td>
<td>95.0 ± 7.8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>159.6 ± 9.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105.2 ± 9.4&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>82.7 ± 9.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>117.0 ± 9.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Glucose (mg dL⁻¹)</strong></td>
<td>94.8 ± 4.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>122.4 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.9 ± 5.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>102.1 ± 2.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>87.8 ± 5.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Insulin (µU mL⁻¹)</strong></td>
<td>29.9 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.9 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.3 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.3 ± 2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.4 ± 2.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>HOMA (arbitrary units)</strong></td>
<td>4.8 ± 0.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18.7 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.1 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5 ± 0.5&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values in a row followed by different letters are significantly different (p < 0.05) by Tukey’s test. *Hibiscus sabdariffa* (HS), *Citrus paradisi* (CP), *Ocimum sanctum* L. (OS).
Table 4. Hepatic markers of oxidative stress: Thiobarbituric acid reactive substance (TBARS), carbonyl content of protein (C=O), catalase (CAT), glutathione peroxidase and glutathione (GPx), and glutathione S-transferase (GST) activities in liver fraction of obese rats treated with infusions. Values are expressed as mean ± SE (n = 7).

<table>
<thead>
<tr>
<th></th>
<th>Healthy control</th>
<th>Obese control</th>
<th>HS</th>
<th>CP</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol mg protein⁻¹)</td>
<td>3.7 ± 0.1ᵇ</td>
<td>21.0 ± 2.8ᵃ</td>
<td>3.3 ± 0.4ᵇ</td>
<td>5.3 ± 1.1ᵇ</td>
<td>3.6 ± 0.3ᵇ</td>
</tr>
<tr>
<td>C=O (nmol carbonyl mg Protein⁻¹)</td>
<td>18.2 ± 0.5ᵈ</td>
<td>34.3 ± 0.3ᵃ</td>
<td>21.5 ± 0.7ᶜ</td>
<td>26.5 ± 1.3ᶜ</td>
<td>23.3 ± 0.7ᶜ</td>
</tr>
<tr>
<td>CAT (μmol min⁻¹ mg⁻¹ protein)</td>
<td>6.1 ± 0.1ᵃ</td>
<td>4.0 ± 0.2ᵇ</td>
<td>6.0 ± 0.4ᵃ</td>
<td>4.5 ± 0.4ᵇ</td>
<td>7.7 ± 0.9ᵃ</td>
</tr>
<tr>
<td>GPx (μmol min⁻¹ mg⁻¹ protein)</td>
<td>12.2 ± 0.2ᵃ</td>
<td>10.4 ± 0.1ᵇ</td>
<td>12.5 ± 0.1ᵃ</td>
<td>11.8 ± 0.1ᵃ</td>
<td>12.5 ± 0.1ᵃ</td>
</tr>
<tr>
<td>GST (μmol min⁻¹ mg⁻¹ protein)</td>
<td>686.3 ± 31.0ᵃ</td>
<td>471.0 ± 27.6ᵇ</td>
<td>588.1 ± 32.9ᵇ</td>
<td>491.4 ± 35.9ᵇ</td>
<td>695.7 ± 40.0ᵃ</td>
</tr>
</tbody>
</table>

Values in a two first row followed by different letters are significantly different with (P≤0.05) by Tukey’s test. Values in enzymatic activity row followed by different letters are significantly different with (P≤0.05) by Dunn's Method. *Hibiscus sabdariffa* (HS), *Citrus paradisi* (CP), *Ocimum sanctum* L. (OS).
Figure 1. Hematoxylin-eosin–stained adipose tissue sections (100x) of obese rats treated with infusions: (A) healthy control, (B) obese control, (C) Hibiscus sabdariffa (HS), (D) Citrus paradisi (CP), and (E) Ocimum sanctum L. (OS) treated group. (F) Adipocytes volume expressed in picoliters (pL). Values are expressed as mean ± SE (n= 7). a,b,c Different letters are significant different (P≤0.05) by Tukey’s test.
Figure 2. Representative photographs of Hematoxylin-eosin–stained hepatic tissue sections (400X): (A) healthy control, (B) obese control, (C) Hibiscus sabdariffa (HS), (D) Citrus paradisi (CP), and (E) Ocimum sanctum L. (OS) treated group.
**Figure 3.** Quantitation of mRNA of hepatic genes involved in fatty acids synthesis of obese rats treated with infusions. (A) SREBP1 and (B) FAS. The geometric mean of SOD and CYCA was used as a reference gene. HS= *Hibiscus sabdariffa*, CP= *Citrus paradisi*, OS= *Ocimum sanctum*. Values are the means ± SE (n = 7). The values for rats treated with infusions were expressed relative to the value for obese rats. Different letters are significant different (P≤0.05) by Tukey’s test.
Figure 4. Quantitation of mRNA of (A) hepatic PPARα and (B) hepatic CPT1α. The geometric mean of SOD and CYCA was used as a reference. HS = Hibiscus sabdariffa, CP = Citrus paradisi, OS = Ocimum sanctum. Values are the means ± SE (n = 7). The values for rats treated with infusions were expressed relative to the value for obese rats. a,b,c,d Different letters are significant different (P≤0.05) by Turkey’s test.