

Safflower Oil (*Carthamus tinctorius* L.) Intake Increases Total Cholesterol and LDL-cholesterol Levels in an Experimental Model of Metabolic Syndrome

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Abstract

Background: Overweight has been considered an important public health problem. To reverse this situation, various types of treatment are proposed. The safflower oil (*Carthamus tinctorius* L.) has been used in the prevention/treatment of obesity.

Objectives: The aim of this study was to evaluate the therapeutic effects of this oil in an experimental model of metabolic syndrome.

Methods: Male Wistar rats initially received a highly palatable (HP) diet for ten weeks for validation of a metabolic syndrome model. Following confirmation, the animals were treated with a HP diet and soybean oil (HPSO) or safflower oil (HPSA) supplementation (1.0 mL/1000 g of animal weight). At the end of the experiment, the body composition, lipid profile and blood glucose levels of the animals were assessed. Student t test was used for statistical analysis.

Results: In the first stage (induction of metabolic syndrome), the animals given the HP diet showed gain weight ($p < 0.001$), visceral adiposity ($p = 0.001$), and significantly higher levels of blood glucose ($p = 0.001$) and triglycerides ($p = 0.03$) than those of the control group. Also, there was a difference in liver weight ($p = 0.01$). These results demonstrate that the HP diet administration is an effective model for the experimental metabolic syndrome study. In the second stage, the animals of the HPSA group showed increased total cholesterol ($p < 0.05$) and LDL-cholesterol ($p < 0.001$) levels.

Conclusion: Under the referred experimental conditions, the use of safflower oil can cause possible deleterious effects on the lipid profile in a metabolic syndrome experimental model. (Int J Cardiovasc Sci. 2017;30(6)476-483)

Keywords: Oils; *Carthamus*; Cholesterol; Metabolic Syndrome; Dyslipidemias; Obesity / prevention & control.

Introduction

Obesity is a chronic, multifactorial disease defined as abnormal or excessive fat accumulation, representing a serious health risk. The prevalence of overweight and obesity is increasing, and 12% of the world population is considered obese.¹ According to Brazilian studies, 52.5% of the adult population is over ideal healthy weight and 17.9% are considered obese. The accumulation of

adipose tissue, particularly visceral fat, can contribute to changes in the body, triggering the metabolic syndrome. This syndrome can be defined as a complex disorder characterized by a set of cardiovascular risk factors related to central fat deposition and insulin resistance.²

In general, the pathophysiological basis for cardiovascular events is atherosclerosis, which is characterized by a chronic inflammatory process of the vascular wall and elevation of circulating serum

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inflammatory markers, such as C-reactive protein (CRP). Regarding dyslipidemia, the primary goal in cardiovascular prevention is to achieve lower LDL-cholesterol (LDL-c) levels, and then reduce triglyceride levels and raise HDL-cholesterol (HDL-c) levels, and this latter goal is considered potentially beneficial for inhibiting the atherothrombotic process.³

To reduce these levels, dietary treatments that require changes in eating habits are proposed.⁴ In this context, the use of safflower oil (*Carthamus tinctorius* L.) is proposed to prevent or treat obesity. Safflower oil is extracted from saffron, a plant belonging to the *Asteraceae* family,^{5,6} contains high content of polyunsaturated (75% linoleic acid) and monounsaturated fatty acids.⁷ This composition stimulated further studies that demonstrated that dietary supplementation with safflower oil had a positive effect on lipid profile, with an antiatherogenic role.

Thus, given the lack of studies on the use of safflower oil on metabolic syndrome, and the existence of conflicting results in the literature, the present study aimed to assess the therapeutic effects of safflower oil on body composition, lipid profile and blood glucose in an experimental model of metabolic syndrome.

Methods

Animals and diet

The animals were provided by the central vivarium of the Health Sciences School of the Federal University of Grande Dourados (UFGD), city of Dourados, Mato Grosso do Sul state, Brazil. The procedures used in this study were approved by the Ethics Committee on Animal Use at UFGD (N° 016/2013), according to the standards established by the Brazilian Committee for the protection of animals used for scientific purposes.⁸

A highly palatable (HP) diet was prepared based on the study by Naderali et al.,⁹ using 33% of ground Labina Purina® food preparation, 33% of condensed milk, 27% of water and 7% of sucrose (2.78 calories/g). The diet was kept under refrigeration until the moment of use, and the normal diet given to the control group was commercial food preparation of the Labina Purina® brand.

The experiment was divided into two stages. In the first stage, an animal model of metabolic syndrome was developed, and, in the second stage, intervention with safflower oil was performed in a disease model similar to the one observed in humans. Male Wistar rats aged 90 days (adult) were used in both experiments.

In the first stage, the animals were divided into two groups (n = 8): one group was fed a normal diet and water *ad libitum*, and the other group was fed a HP diet and water *ad libitum*, for ten weeks. In the second stage, the animals were also divided into two groups (n = 5-6), and both groups were fed a HP diet and water *ad libitum*, for ten weeks. The HP diet and soybean oil (HPSO) and HP diet and safflower oil (HPSA) were given by oral gavage to the animals. The daily administered dose was 1.0 mL/1000 g of animal weight.^{10,11}

The safflower oil (Galena®) and soybean oil (Liza®) used in this study were purchased at a local store in the city of Dourados.

The same parameters were assessed in both stages. Therefore, food consumption and animal weight were daily measured. After ten weeks of treatment, the animals were fasted for 12 hours, anesthetized with xylazine and ketamine (10 mg/kg; 75 mg/kg) and euthanized by exsanguination via the inferior vena cava. The blood samples were centrifuged at 3000rpm for 15 minutes, and the serum was kept in freezer at -20°C until analysis. Adipose tissue samples from five sites was removed and weighed. The livers were subjected to histological analysis for the presence of hepatic steatosis.

Fasting blood glucose

For blood glucose assessment, the animals were fasted for 16 hours, and glucose was measured in blood collected from the tail artery, by using a glucometer (*Testline Stripts125*), according to Formagio et al.¹¹

Concentration of total cholesterol, HDL-c, LDL-c and triglycerides

The determination of total cholesterol, HDL-c and triglycerides in serum was based on the colorimetric enzymatic method, using commercial kit (*Labtest*®), according to the manufacturer's instructions. The results were obtained with a spectrophotometer (*Aaker/Bel - SF325NM - S05*®). The concentration of LDL-c was obtained with a formula, according to the instructions of the manufacturer of the kit for quantification of HDL-c, using the Friedewald equation, where: $VLDL-c = Triglycerides / 5$ and $LDL-c = Total\ Cholesterol - (HDL-c + VLDL-c)$.

Liver weight

The extracted liver was immediately immersed in saline solution to remove excess blood and then dried on paper and weighed.

Weight of adipose tissue

Adipose tissue samples from five sites (epididymal, retroperitoneal, perirenal, mesenteric and omental) were removed and weighed, and subsequently the animal fat (percentage of adipose tissue in each site to final body weight) was determined.

Liver histology

After euthanasia, the liver was removed for histological analysis and fixed in 10% formaldehyde solution until it was embedded in paraffin. Then, microtome sections, 7 μm thick, were made and then mounted on glass slides. Each slide had a total of four sections removed from the paraffin block. The slides were then stained with hematoxylin and eosin, and analyzed under the microscope coupled to a digital camera with 200x magnification, for the classification of the presence of steatosis, according to Abbas et al.¹² and Alves and Mello.¹³

Statistical analysis

The results were expressed in mean \pm standard error of mean (SEM), and analyzed with Prisma 5.0 software (GraphPad Software, USA). Student *t* test was used to assess whether the means of the groups were statistically significant, and $p < 0.05$ values were considered statistically significant.

Results

The results of the first stage of the study where we attempted to develop an animal model of metabolic

syndrome can be seen in Table I. There was no statistically significant difference between the groups regarding water intake (unreported data).

In the second stage, the animals were fed a HP diet (that induces metabolic syndrome) supplemented with soybean oil (HPSO) or safflower oil (HPSA). Food and water intake of the animals was measured. However, there was no statistically significant difference between the groups for these parameters (unreported data).

Significant changes were observed in the lipid profile of the animals, and the HPSA group showed a higher concentration of total cholesterol and LDL-c than the control group (HPSO) (Figures 1A and 1B, $p = 0.03$ and $p = 0.001$, respectively). In contrast, HDL-c and triglycerides did not statistically differ between the groups (Figures 1C and 1D).

The HPSA group showed lower levels of fasting blood glucose as compared to the control group, but this difference was not statistically significant (Figure 2).

According to our results, there was no statistically significant difference regarding liver weight and weight gain between the control group and the HPSA group (Figures 3 and 4A, respectively). However, the HPSA group showed higher values of total adipose tissue, and epididymal and visceral adiposity, which were not statistically significant though (Figures 4B, 4C and 4D, respectively).

Regarding histological analysis of the liver, groups HPSO and HPSA showed mild microvesicular steatosis and moderate micro- and macrovesicular steatosis, respectively (Figure 5).

Table 1 – Total dietary intake, body composition, liver weight and biochemical parameters in rats fed with a normal diet and a highly palatable diet

	Control	Highly Palatable
Dietary intake (calories/group/week)	2606 \pm 33.32	3084 \pm 66.56 ***
Visceral Adiposity (%)	4.5 \pm 0.3	6.401 \pm 0.3 ***
Liver weight (g)	11.3 \pm 0.4	12.8 \pm 0.4 **
Total cholesterol (mg/dl)	82.8 \pm 13.2	86.2 \pm 7.1
Triglycerides (mg/dl)	96.6 \pm 13.1	150.2 \pm 19.0 *
Fasting blood glucose (mg/dl)	73.7 \pm 1.4	85.6 \pm 2.2 ***

Values expressed in mean \pm standard error of mean (SEM). Student *t* test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $n = 8-9$.

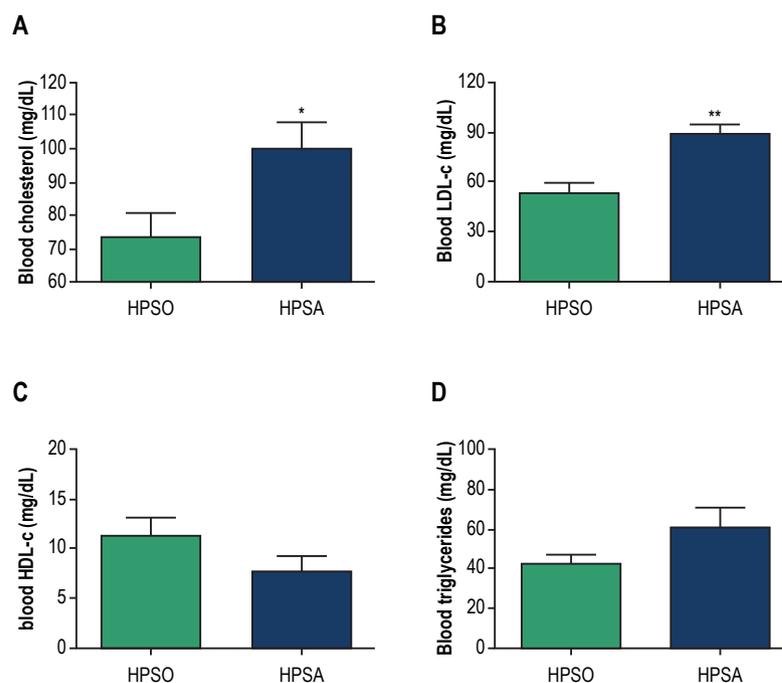


Figure 1 – Concentration of total cholesterol (A), LDL-cholesterol (B), HDL-cholesterol (C) and triglycerides (D) of rats fed with a highly palatable (HP) diet for 10 weeks, supplemented with soybean oil (HPSO) or safflower oil (HPSA). $n = 5-6$. Values expressed in mean \pm standard error of mean (SEM). Student t test. * $p < 0.05$; ** $p < 0.01$.

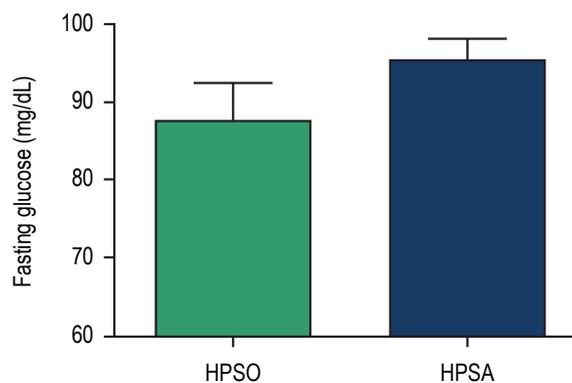


Figure 2 – Fasting blood glucose of rats fed with a highly palatable (HP) diet for 10 weeks, supplemented with soybean oil (HPSO) or safflower oil (HPSA). $n = 5-6$. Values expressed in mean \pm standard error of mean (SEM). Student t test.

Discussion

Several experimental models have been proposed for the study of obesity and its metabolic disorders, most of them based on genetic modifications.^{14,15} However, the animal model that most resembles the etiology of human obesity is the one involving high

intake of dietary fat.¹⁶ However, the extrapolation of many of these animal models to human obesity is limited by the difficulty in reproducing disorders that characterize metabolic syndrome, such as central obesity and changes in the metabolism of lipids and glucose. Also, they require long periods of time for the induction of obesity.^{17,18} Thus, the model used in this

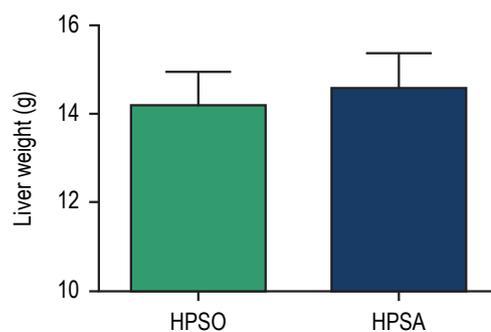


Figure 3 – Liver weight of rats fed with a highly palatable (HP) diet for 10 weeks, supplemented with soybean oil (HPSO) or safflower oil (HPSA). $n = 5-6$. Values expressed in mean \pm standard error of mean (SEM). Student t test.

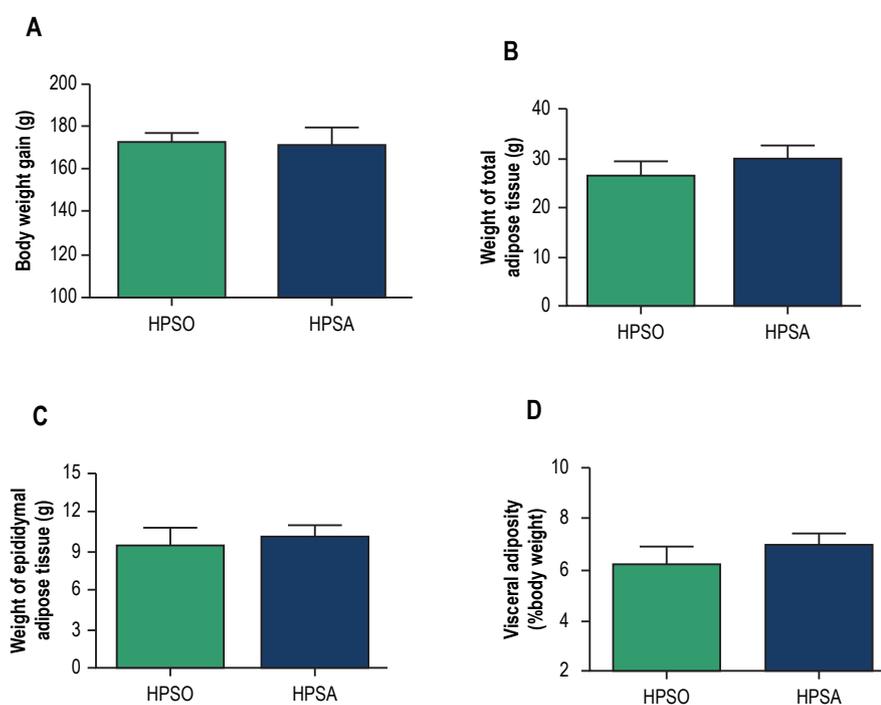


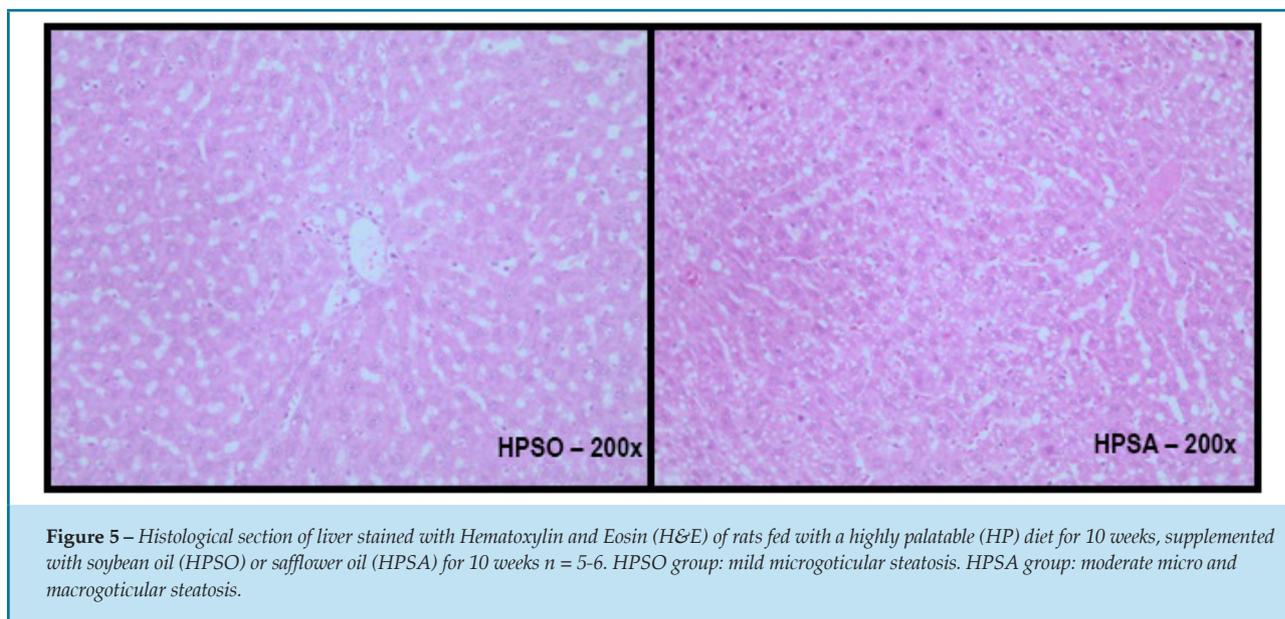
Figure 4 – Body weight gain (A), weight of total adipose tissue (B), epididymal adipose tissue (C) and visceral adiposity (D) of rats fed with a highly palatable (HP) diet for 10 weeks, supplemented with soybean oil (HPSO) or safflower oil (HPSA). $n = 5-6$. Values expressed in mean \pm standard error of mean (SEM). Student t test.

study is the most suitable for the research of metabolic syndrome interventions, because it effectively mimics the disorder observed in humans, and requires a relatively lower induction time compared to other studies,^{19,20} which reduces research costs.

Such findings (visceral obesity, hyperglycemia and hypertriglyceridemia) are consistent with the diagnosis of metabolic syndrome, also including hepatic steatosis.

Several studies indicate an association between obesity and metabolic disorders, particularly the intake of hypercaloric and high carbohydrate diets, which is also demonstrated in our study.¹⁶⁻²¹

According to the results obtained in the second stage of the study, there was no statistically significant difference between the groups regarding weight gain, which is similar to the findings obtained in a study by



Fernandes et al.,²² where no significant difference was found in the weight gain of animals in the groups given a diet supplemented with 1% of conjugated linoleic acid (CLA) compared to the groups that were not given the referred supplement. This finding is also corroborated by the study by Bhattachaya et al.,²³ where BALB/C mice fed with a high-fat diet supplemented with 0.4% of CLA showed no change in body weight.

Nevertheless, different results were obtained by DeLany et al.¹⁹ and Park and Pariza.²¹ According to these studies, changes in body composition following the supplementation of CLA may be related to decreased proliferation and differentiation of preadipocytes, elevation of energy expenditure and changes in the concentration of leptin, a protein synthesized and secreted by adipocytes, involved in energy homeostasis. In contrast with the suggestions of these studies, in our study the group supplemented with safflower oil showed higher visceral adiposity, although this difference was not statistically significant. This fact indicates that there are conflicting reports in the literature regarding changes in body composition associated with the use of safflower or its main component, CLA.

Analysis of the lipid profile of the animals showed that the HPSA group had a higher concentration of total cholesterol and LDL-c compared to the control group (HPSO). Similar results were obtained by Fernandes et al.,²² who demonstrated increase in cholesterol levels following supplementation with 1% of CLA. This fact can be explained by changes in

hepatic lipid metabolism caused by CLA, which may affect metabolic interconversion of fatty acids in the liver, resulting in a modified composition of fatty acids, causing changes in lipoproteins.²⁴

Still regarding cholesterol, in the above-mentioned study by Fernandes et al.²² with animals supplemented with CLA distributed into active and sedentary, there was improvement in HDL-c levels in the sedentary group supplemented with CLA. However, in our study, as well as in the study by Marques et al.,²⁵ which used safflower oil other than CLA, there was no statistically significant difference between the groups regarding HDL-c and serum triglycerides.

In turn, analysis of fasting blood glucose showed that the HPSA group had higher values compared to the control group, though this difference was not statistically significant. Marques et al.²⁵ concluded that exclusive supplementation with 2% CLA affected the metabolism of glucose in mice, and these animals had severe hyperglycemia compared to the control group. This difference may not have been observed in our study because we used safflower oil, not isolated CLA.

The study by Tanaka et al.²⁶ indicate that supplementation with CLA induces insulin resistance in animals and humans. However, other studies had controversial results, such as the study of Parra et al.,²⁷ these authors demonstrated that the mixture of CLA isomers did not increase insulin resistance in rats given oral supplementation.

Therefore, there is no consensus on the relationship between CLA intake and insulin sensitivity. Further studies are needed to clarify such metabolic changes.

Also, there were no differences in the groups regarding the relative liver weight. The study by Gaiva et al.²⁸ with rats fed diets rich in polyunsaturated fatty acids reported increased liver weight. It can be suggested that in our study there was no statistically significant difference in liver weight between the groups because soybean oil and safflower oil are both sources of polyunsaturated fatty acids, and, thus, have the same action on liver.

However, analysis of liver histological sections showed that supplementation with safflower oil interferes with hepatic steatosis, and the presence of micro- and macrovesicular steatosis is noticeable in this group. According to Reddy,²⁹ fatty acid synthesis is stimulated to promote the storage of energy substrate in adipose tissue, a process that affects lipid metabolism, especially oxidation homeostasis and lipid synthesis, resulting in obesity, resistance to insulin, dyslipidemia and hepatic steatosis.

Conclusion

Our results showed that animals fed a HP diet constitute an effective model for the experimental study of metabolic syndrome, due to its similarity with the etiology of the syndrome observed in humans, and also because that model does not require the use of genetically modified animals, has shorter induction time and relatively low costs.

We concluded that the effect of safflower oil on the prevention of adverse impacts on body composition, lipid

profile and blood glucose was not confirmed under the conditions of the present study. Instead, supplementation with safflower oil resulted in the elevation of total serum cholesterol and its most atherogenic fraction, LDL-c, as well as in hepatic steatosis, indicating that the use of safflower oil may have deleterious effects on lipid profile and health.

Author contributions

Conception and design of the research: Santana LF, Dutra TS, Freitas CF, Soares FLP. Acquisition of data: Santana LF, Dutra TS, Souza MA, Freitas CF, Oesterriech AS, Kassuya CPL, Soares FLP. Analysis and interpretation of the data: Santana LF, Souza MA, Freitas CF, Oesterriech AS, Kassuya CPL, Soares FLP. Statistical analysis: Santana LF, Souza MA, Freitas CF, Oesterriech AS, Kassuya CPL, Soares FLP. Writing of the manuscript: Santana LF, Freitas CF, Kassuya CPL, Soares FLP. Critical revision of the manuscript for intellectual content: Santana LF, Freitas CF, Kassuya CPL, Soares FLP.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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