The epididymal, retroperitoneal and liver tissues were weighed. Hepatic tissue were also used to quantification of lipids. The results were analyzed using Student’s T test ($p < 0.05$) and expressed as mean ± SE. A progressive decrease in the accumulation of lipids in adipose tissues and in the liver, as well as in the serum levels of TAG and VLDL was observed between the HFP8 vs HFP4 groups. The carbohydrate metabolism presented very expressive changes in a short time of treatment (HFP4). The data suggest that MC appears to be effective in preventing changes in metabolism, probably by preventing the increase of lipid deposits, decreasing the inflammatory process and leading to an improvement in insulin sensitivity.

Key words: Obesity; Adipose tissue; Insulin resistance; Fatty liver; Momordica charantia

INTRODUCTION

People affected by metabolic disorders have increased steadily in recent decades. Among these changes, overweight and obesity have taken on alarming proportions. International studies estimate that 1 billion people are overweight, with approximately 500 million considered obese[1]. Obesity, characterized mainly by the accumulation of fat in the adipose tissue, can cause several compromises in the body, such as hypertension, dyslipidemia, hyperglycemia and hyperinsulinemia[2]. In addition to the excess of stored lipids, obesity is also seen as a low-grade inflammatory process. Adipose tissue is able to release cytokines (such as TNF-α and interleukin-6) that are known to disrupt organic homeostasis. For example, these cytokines may interfere with the action of insulin and trigger pathologies such as Insulin Resistance (IR), Diabetes Mellitus (DM) and Non-Alcoholic Fatty Liver Disease (NAFLD)[3].

In general, treatment for obesity-related metabolic disorders is based on changes in lifestyle, including exercise and improved eating habits[4]. The administration of medicines is also essential. Among
them, those that improve insulin action, as well as fat absorption in the body, such as metformin, thiazolidinediones, orlistat and simvastatin are commonly used[6]. However, these medications have several side effects, making it extremely important to seek alternative therapeutic forms. In this context, we decided to evaluate *Momordica charantia* (MC) as a new potential intervention for metabolic disorders. MC is a popular and nutritious healthy vegetable used mainly in Asian countries. Phytochemical studies with the species have identified a series of secondary metabolites with bioactive properties, mainly steroids, alkaloids, catechins and saponins[9]. Research indicates that MC stimulates β-oxidation and translocation of GLUT 4 to the cell membrane by activation of the AMPK protein (Adenosine Monophosphate-activated Protein Kinase) and that its hypoglycemic action can also be mediated by FGF 21 (Fibroblast Growth Factor 21). This factor acts as an endocrine hormone decreasing insulin resistance, triacylglycerol concentration and body weight[7].

For thus, the objective of this study was to investigate the effect of *Momordica charantia* tea to prevent metabolic biochemical changes in carbohydrate and lipid metabolism in mice fed a high fat diet. In this way, we intend to put forward a more accessible and less toxic therapeutic alternative for patients who present metabolic alterations so as to provide them a higher life quality.

**METHODOLOGY**

**Animals and Treatments**

Swiss male mice (n = 6-8 animals/group) with initial weight varying from 16 to 18 grams were used. The animals were divided into experimental groups and kept in cages in the Laboratory of the Metabolic Biochemistry of the Federal University of São João del-Rei with controlled temperature of 25°C ± 2°C and a light-dark cycle of 12 hours. The groups were: C4 (control diet - normolipid and normocaloric - and water ad libitum for 4 weeks), C8 (control diet - normolipid and normocaloric - and water ad libitum for 8 weeks), CP4 (control diet - normolipid and normocaloric - and MC tea for 4 weeks), CP8 (control diet - normolipid and normocaloric - and MC tea for 8 weeks), HF4 (high fat diet and water ad libitum for 4 weeks), HF8 (high fat diet and water ad libitum for 8 weeks), HFP4 (high fat diet and MC tea for 4 weeks) and HFP8 (high fat diet and MC tea for 8 weeks). Treatment times were chosen due to the observation of acute and chronic attenuations of MC tea.

The palatable hyperlipidic diet consists of commercial rat chow plus peanuts, milk chocolate, and sweet biscuits in the proportion of 3:2:2:1. All components were powdered and mixed. This diet was composed of 20% protein, 20% fat, and 40% carbohydrate. The control diet contained 20% protein, 4.5% fat, and 55% carbohydrate. The caloric densities of the diets were determined using an IKA-C400 adiabatic calorimeter. The caloric density was 21.40 kJ/g (35% of calories from fat) for the palatable hyperlipidic diet and 17.03 kJ/g for the chow diet[9]. After the treatment period of each group in their respective diets, the animals were submitted to the experiments.

The experimental protocol was approved by the Institution’s ethics committee by Prof. Leila of Genova Gaya on May 29, 2015 (CEUA No. 014/2015)

**Tea preparation of Momordica charantia**

MC tea was prepared from commercially purchased aerial parts of the plant (Cha & Cia) and was prepared according to manufacturer’s directions: one liter of water was boiled with two tablespoons of the herb and after the solution reached the boiling, the system was mulled for 10 minutes. Later the tea was sifted, cooled and served to the mice.

**Body weight, water and food intake**

Body weight (g) was monitored by weighing all animals daily. Daily water (mL) and food intake (g) was estimated from the difference between the quantity of water and feed offered, subtracting the quantity disregarded.

**Glucose Tolerance Test (GTT)**

Mices HF4, HFP4, HF8 and HFP8 groups were fasted for 6 hours and subsequently administered with 2 g of glucose/kg body weight intraperitoneally. Blood glucose levels were measured using a glycosometer at time 0 (before administration) and 30, 60, 90 and 120 minutes after glucose administration. Results were expressed as mg/dL.

**Sample collection**

The animals were anesthetized intraperitoneally by ketamine 10% (0.01 mg/g) and xylazine 2% (0.1 mg/g) injection. Blood samples were collected by intracardiac puncture. After blood collection, the animals were euthanized by cervical dislocation. The adipose retroperitoneal and epididymal tissues, as well as liver, were collected and weighed. A portion of the liver was used for lipid quantification.

**Extraction and quantification of liver lipids**

Approximately 1g of liver was weighed and homogenized in 10 ml of chloroform/methanol solution (2:1) remaining standing for extraction. The next day, 0.9% saline was added to separate the insoluble part in the ratio of 2 mL to 10 mL of the filtrate. After separation of the phases, an aliquot of the chloroform part was transferred to a pre-weighed vessel and the fat (total lipids) resulting from the evaporate was quantified by gravimetry[9]. Hepatic cholesterol and triacylglycerol concentrations were determined by means of diagnostic kits (LabTest) after suspension of fat obtained previously in 2 ml of isopropanol. The results were expressed in mg/100g of liver.

**Serum biochemical dosages**

Quantification of of glucose, triglycerides, total cholesterol, HDL cholesterol and ALT (Alanine Aminotransferase) were performed after blood collection, using diagnostic kits from Labtest. The mice were not fasted. The VLDL fraction was calculated by the Friedwald equation, where: VLDL = TAG ÷ 5. All results are expressed in mg/dL.

**Statistical analysis**

For the statistical analysis of the results obtained in the experiments, the GraphPad Prism 7.0 Demo® Software was used. A normality test was performed and, afterwards, the Student’s T test was applied comparing all the groups. The results were expressed as mean ± Standard Error and considered as significant p < 0.05.

**RESULTS AND DISCUSSION**

Initially, the consumption of water and animal feed was evaluated throughout the treatments, as well as the evolution of body weight. During the whole life of the animals, it was possible to observe a decrease in the food intake between the HF4 / HF8 in relation to the C4 / C8 (Figure 1a). There was a decrease in water consumption from the second week between HF4 / HF8 and C4 / C8 (Figure 1b). Regarding body weight, there was an increase (~ 10%) between HF8 compared to C8 in the last week. Treatment with MC (HFP8) was able to promote weight reduction from the fifth week compared to HF8 (~ 12%, ~ 15%, ~ 15%, ~ 17%, respectively) (Figure 2).

High Fat Diets are associated with lower food intake[10]. Thus, the
Food (a) and water (b) intake of the animals throughout the treatments. C (control), CP (control treated with MC), HF4/HF8 (hyperlipidic/hypercaloric diet for 4 and 8 weeks) and HFP4/HFP8 (hyperlipidic/hypercaloric diet treated with MC tea for 4 and 8 weeks). Results expressed as mean ± standard error (n = 6-8 animals per group). Being (p<0.05) a HF4 vs C4, b HF8 vs C8.

Figure 2 Body weight of the animals throughout the treatments. C (control), CP (control treated with MC), HF4/HF8 (hyperlipidic/hypercaloric diet for 4 and 8 weeks) and HFP4/HFP8 (hyperlipidic/hypercaloric diet treated with MC tea for 4 and 8 weeks). Results expressed as mean ± standard error (n = 6-8 animals per group). Being (p<0.05) a HF8 vs C8, b HF8 vs HF4.

Animals fed a high fat diet develop an accumulation of triacylglycerol in hepatocytes. It could happen due to the large amount of free fatty acids that enter the liver which are originated from the adipocytes. Liver can become overloaded and so it is unable to process the excess and transport it to other tissues through VLDL. However, the lipid-lowering effects caused by MC may also be related to increased lipid oxidation caused by the presence of this work lead to the accumulation of fats in different parts of the body when compared to normocaloric diets, especially in the epididymal and retroperitoneal adipose tissues. This is because normally these tissues are already sites of lipids accumulation that cannot be secreted in the body. Adipose tissue is a dynamic endocrine organ that produces several substances (adipokines), such as resistin and interleukins, which contribute to the establishment of an inflammatory process. MC has components such as steroids, triterpenoid glycosides, alkaloids, flavonoids, polyphenols, carotenoids, fatty acids, and some insulin-like polypeptides that can reduce both insulin resistance and fat deposition caused by obesity.

When differences in liver weight were observed, unexpectedly no increase in high fat groups was found compared to their respective controls. It is likely that the great variation in the body weight of the experimental groups may therefore influence in the weight of the organs. Anyhow, since a significant decrease in HFP8 vs HF8 was observed, the data together reaffirm the effectiveness of MC treatment in preventing progressive changes in the accumulation of fat deposition in tissues.

Evaluating the total lipids present in the liver, a progressive decrease of lipids in C8 vs C4 (~63%) and in CP8 vs CP4 (~55%) was observed. The HF8 group showed an increase compared to C8 (~60%), but a decrease compared to HF4 (~36%). There was an increase in total lipids in HFP8 vs HF8 (~87%). Hepatic triacylglycerol levels were lower in C8 vs C4 (~40%) and were higher in HF8 vs C8 (~170%) and HFP4 vs HF4 (~43%). Hepatic cholesterol levels were lower only in HF8 vs HF4 (~26%). Finally, it has been found that MC tea administration was able to decrease hepatic TAG and CTO levels of the HFP8 vs HFP4 groups progressively by approximately 40% (Table 2).

Evaluating the lipid profile present in the blood, there was an increase of CTO in the HF4/HF8 compared to the C4/C8 (~50% and ~35%, respectively). TAG levels were decreased in the C8 (~34%) vs HF8 (~60%) and HFP8 vs HF8 (~43%). Very Low Density Lipoprotein (VLDL) was decreased in several groups: CP4 vs C4 (~33%), CP8 vs CP4 (~30%), HF4 vs C4 (~53%), HF8 vs C8 (~60%) and HFP8 vs HF4 (~43%). There was no difference in the levels of high-density lipoprotein (HDL) in any of the groups analyzed. MC tea administration was responsible to decrease TAG and VLDL levels between the HFP8 vs HF4 progressively by approximately 60% (Table 3).

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**Table 1** Liver weight (g), epididymal (EPD) and retroperitoneal (RET) tissues (g) of the animals after the treatments.

| Groups | Liver (g) | EPD | RET |
|--------|-----------|-----|-----|
| C4     | 2.438 ± 0.15 | 0.962 ± 0.19 | 0.409 ± 0.08 |
| C8     | 2.460 ± 0.04 | 0.735 ± 0.13 | 0.278 ± 0.02 |
| CP4    | 2.310 ± 0.01 | 1.030 ± 0.09 | 0.278 ± 0.03 |
| CP8    | 2.237 ± 0.05 * | 0.618 ± 0.09 * | 0.193 ± 0.02 * |
| HF4    | 1.828 ± 0.05 * | 1.090 ± 0.17 | 0.352 ± 0.05 * |
| HF8    | 1.939 ± 0.07 | 2.240 ± 0.27 * | 0.822 ± 0.07 * |
| HFP4   | 1.960 ± 0.16 | 1.414 ± 0.23 | 0.624 ± 0.11 |
| HFP8   | 1.623 ± 0.13 * | 1.476 ± 0.25 * | 0.520 ± 0.07 * |

Results expressed as mean ± standard error (n = 6-8 animals/group). Being (p < 0.05) * difference between 4 and 8 weeks in the same group, 1 HFP4 vs C4, 2 C8 vs CP8, 3 HFP8 vs HF8.

**Table 2** Total lipids (LT), triacylglycerol (TAG hep.) and hepatic cholesterol (CTO hep.) of the animals after the treatments. C (control), CP (control treated with MC), HF4/HF8 (hyperlipidic/hypercaloric diet for 4 and 8 weeks) and HFP4/HFP8 (hyperlipidic/hypercaloric diet treated with MC tea for 4 and 8 weeks).

| Groups | LT (g/100g) | TAG hep. (mg/100g) | CTO hep. (mg/dL) |
|--------|-------------|--------------------|-----------------|
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**Table 3** Serum levels of total cholesterol (CTO), triacylglycerol (TAG), VLDL and HDL after treatment. C (control), CP (control treated with MC), HF4/HF8 (hyperlipidic/hypercaloric diet for 4 and 8 weeks) and HFP4/HFP8 (hyperlipidic/hypercaloric diet treated with MC tea for 4 and 8 weeks).

| Groups | CTO (mg/dL) | TAG (mg/dL) | VLDL (mg/dL) | HDL (mg/dL) |
|--------|-------------|-------------|-------------|-------------|
| C4     | 7.484 ± 3.65 | 367.607 ± 14.87 | 73.523 ± 2.97 | 21.85 ± 2.19 |
| C8     | 8.837 ± 3.22 | 244.217 ± 12.71 * | 48.843 ± 2.54 | 37.69 ± 3.19 |
| CP4    | 6.912 ± 6.84 | 248.063 ± 12.08 * | 49.612 ± 2.41 * | 57.03 ± 10.23 |
| CP8    | 10.632 ± 17.41 | 175.483 ± 35.26 | 35.101 ± 7.05 * | 53.773 ± 4.42 |
| HF4    | 112.168 ± 8.25 * | 174.295 ± 6.61 * | 34.860 ± 1.31 * | 63.08 ± 2.8 |
| HF8    | 110.537 ± 5.24 * | 99.592 ± 15.41 ** | 19.960 ± 3.08 ** | 64.41 ± 7.27 |
| HFP4   | 119.841 ± 10.69 | 200.564 ± 21.36 | 40.114 ± 4.27 * | 44.167 ± 9.81 |
| HFP8   | 118.816 ± 5.26 | 80.216 ± 7.41 * | 16.631 ± 1.27 * | 60.751 ± 6.98 |

Cholesterol (CTO) (mg/dL), triacylglycerol (TAG) (mg/dL), VLDL (mg/dL) and HDL (mg/dL). Results expressed as mean ± standard error (n = 6-8 animals/group). Being (p < 0.05) * difference between 4 and 8 weeks in the same group, 1 vs C4, 2 vs C8.

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Figure 3 Serum Alanine Aminotransferase (ALT) levels of the animals after the treatments. HF4/HF8 (hyperlipidic/hypercaloric diet for 4 and 8 weeks) and HFP4/HFP8 (hyperlipidic/hypercaloric diet treated with MC tea for 4 and 8 weeks). Results expressed as mean ± standard error (n = 6-8 animals per group). Being (p < 0.05) * difference between 4 and 8 weeks in the same group and ** vs HF8.

Figure 4 Glucose Tolerance Test (a) and serum glucose levels of the animals after the treatments. C (control), CP (control treated with MC), HF4/HF8 (hyperlipidic/hypercaloric diet for 4 and 8 weeks) and HFP4/HFP8 (hyperlipidic/hypercaloric diet treated with MC tea for 4 and 8 weeks). Results expressed as mean ± standard error (n = 6-8 animals per group). Being (p < 0.05) * HF4 vs HF4, 1 HF8 vs HF4, 2 HFP4 vs HF8 vs HFP8 vs HF8 vs HFP4 * difference between 4 and 8 weeks in the same group ** vs C4 and *** same group HF.

MC has a fundamental action in the fight against the accumulation of fats in the blood[20]. An interesting fact was that the HFP4 group had higher levels of hepatic TAG when compared to the untreated group in the same period. One hypothesis is that initially the plant stimulates the stocking of fats to the liver in order to lower blood levels. Maybe, only after a longer period MC would be able to oxidize these lipids. However, in the lipid metabolism, there were statistically observed progressive decreases in the accumulation of lipids, both in the liver and in the blood, demonstrating that the substances present in the MC act in a way to decrease the picture of the polyphenols the plant contains[19]. There was a progressive decrease in VLDL levels over the weeks between treatment groups. The main function of this lipoprotein is to transport endogenous products like phospholipids, triglycerides and cholesterol into the bloodstream. When this lipoprotein falls into the bloodstream, it turns into LDL, which can be dangerous. LDL promotes cholesterol accumulation in cells, which leads to vessels and clogging[19]. Therefore, there is a hypothesis that LDL levels would decrease progressively over the course of treatment.

The data presented, taken together, lead us to consider that...
hepatic steatosis and dyslipidemias caused by an excess of fats[27].

Previous studies with MC have demonstrated that this plant stimulates the activation of Peroxisome Proliferator-Activated Receptors (PPARs), especially the PPARα and PPARγ subtypes, which facilitate lipid metabolism, reducing blood lipids and the inflammatory process[22]. The decrease of the fat accumulation caused by the plant can consequently result in weight loss effect[27]. Molecular mechanisms regarding the PPARs action may implicate in inhibition of cytokines secreted by monocytes leading to the expression of inflammatory genes such as IL-1, IL-2, IL-6, IL-8, TNF-α and metalloproteinases (MMPs)[24]. PPARs agonists such as fibrates have been used for a period in the treatment of dyslipidemias, such as atherosclerosis[27].

After analysis of lipid metabolism, levels of Alanine Aminotransferase (ALT) were measured. It is known that this enzyme is well representative as evidence of hepatic damage since it is present within the hepatocytes and whenever cell injury occurs, it reaches the bloodstream making its serum levels able to be quantified[28]. As a result, ALT levels were shown decreased in HFP8 vs HF8 (~ 45%) and HFP8 vs HFP4 (67%) (Figure 3). The decrease of the enzyme in these groups may be related to a greater protection of the liver against liver injury. In this way, as it was also observed a lower weight of this organ, it is possible that the inflammatory process of the liver cells is smaller in the HFP8 group than the other groups. One of the potential effects of MC has been the reduction of oxidative stress, reducing the infiltration of inflammatory cells and subsequent fibrosis[27]. This improvement also acts to prevent hepatocyte damage by decreasing the release of this enzyme into the bloodstream[28].

Carbohydrate metabolism was evaluated in two steps: first through the Glucose Tolerance Test (GTT) and, after euthanasia, the analysis of glucose serum levels. The GTT results showed that glucose levels were increased in the HFP4 vs HF4 group (~ 35%) during the initial time. Within 30 minutes after glucose administration there was an increase in the HFP8 vs HF4 group (~ 25%). It appears that MC allowed an improvement in glucose uptake at the end of the experiment, as there was a decrease in their levels in the in the HFP8 vs HF8 (~ 30%). Progressively, tea was able to lower glycemia in the HFP8 vs HF4 group (~ 44%) (Figure 4a).

Finally, serum glucose levels were increased between groups C8 vs C4 (~ 87%), C8 vs C4 (~ 74%), HF4 vs C4 (~ 45%) and HF8 vs HFP4 (~ 53%). MC treatment was efficient in preventing glucose increase, as there was a decrease in their levels in the HFP4 vs HF4 (~ 30%) and HFP8 vs HF8 (~ 17%). However, there was an increase in HLP8 vs HLPF4 (~ 84%) (Figure 4b).

Studies show that the administration of the tea containing the extract of MC seeds regulated mainly the insulin-signaling pathway in muscles and adipose tissues. This mechanism can be explained by the activation of two key elements in carbohydrate metabolism: FGF21 and AMPK[27]. FGF21 has been discovered as a new type of cytokine that regulates the glucose and lipids metabolism. In addition, FGF21 acts as an hepatic endocrine factor that promotes thermogenic activity and modulates lipid metabolism by acting as an adipokine, promoting the uptake of glucose in adipocytes[27]. Therefore, it is suggested that it may act against pathologies present in the liver and in metabolic disorders such as, for example, the improvement of insulin action in patients with Diabetes Mellitus 2 (DM2). However, it is reported that the accumulation of fat in the liver in animals of the HF groups, caused by excess lipids in the diet, can negatively modulate FGF21 signaling through an increase in resistance to this growth factor. It means that, obese animals consequently show insulin resistance and increase in glucose levels[27].

Some bioactive compounds present in the MC such as momordina, xanthine, triterpenoids, and cucubitans glycocide have the ability to stimulate GLUT4 translocation to the cell membrane by activation of the AMPK pathway in adipocytes[27]. The AMPK system is a cell energy sensor, activated by high concentrations of ADP and aims to maintain organic homeostasis. This protein consists of a serine-threonine kinase heterotrimERIC expressed in different tissues, including the brain, liver and skeletal muscle[27]. Specifically, when AMPK phosphorylation occurs, this protein is activated and through a signaling pathway ends up forming vesicles in the cytosol, which carry the glucose transporters (GLUTs) to the plasma membrane. Studies have shown that rats fed a high fructose diet and MC treated had increased AMPK phosphorylation levels when compared to the untreated group, leading to an improvement in insulin action and oxidative capacity of the cells[28].

CONCLUSION

Taken together, it is noted that MC tea was able to prevent increased levels of glucose, body weight and adipose tissue. Due to the presence of FGF21 and other hypoglycemic compounds, it may act improving the interaction of insulin with cellular receptors, enhancing glucose transporters action (GLUTs), and causing glucose to be captured into the cell and used as energy. Another effect of MC is to decrease the levels of adipokine release, in addition to fight the oxidative damage caused by a hyperlipidic diet, thereby reducing the inflammatory effect. Furthermore, it stimulates β-oxidation of lipids stored in adipose tissue, inducing TAG to be consumed and to generate energy within the cell. All these mechanisms occurring due MC effect, mainly in the liver, can contribute to the improvement of lipid and glucose metabolism in animals fed with a high lipids diet.

ACKNOWLEDGMENTS

UFSJ and CNPq (National Council for Scientific and Technological Development).

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