Evaluation of biochemical markers in diabetic rats fed diets supplemented with fruit purees

Eduardo Mendeleev Becerra-Verdín, Úrsula Mireya Morales Ávila, Hugo Sergio García-Galindo, Rubén Montalvo-González, Alfonso Castañeda-Martínez and Efigenia Montalvo-González

ABSTRACT
In this work, the effect of intake of fruit purees (guava-strawberry, guava-blackberry, guava-soursop, guava-passion fruit) by diabetic rats on reduction of plasma glucose and inflammation biomarkers was evaluated. Consumption of fruit purees in addition to a standard diet was associated with a reduction in body weight (20%), control of glucose metabolism, by decreasing plasma glucose (62.1%). We found a significant reduction in activities of alanine aminotransferase (49.41%), alkaline phosphatase (57.25%) and \( \gamma \)-glutamyl transferase (76%), which are useful markers of inflammatory process in the liver. Purees did not alter lipid metabolism and a remarkable improvement in liver morphology was observed. The diets order with the best beneficial effects was diet supplemented with guava-blackberry > guava-strawberry > guava-passion fruit > guava-soursop fruit. Consumption of fruit purees represent a therapeutic alternative by diabetic individuals.

Evaluación de marcadores bioquímicos en ratas diabéticas alimentadas con dietas suplementadas con purés de frutas

RESUMEN
En este trabajo, se evaluó el efecto de la ingesta de purés de frutas (guayaba, guayaba, mora, guanábana, guayaba y maracuyá) en ratas diabéticas sobre la reducción de la glucosa plasmática y biomarcadores de inflamación. El consumo de purés de fruta además de una dieta estándar se asoció con una reducción en el peso corporal (20%), control del metabolismo de la glucosa, al disminuir la glucosa en plasma (62,1%). Encontramos una reducción significativa en las actividades de alanina aminotransferasa (49,41%), fosfatasa alcalina (57,25%) y \( \gamma \)-glutamil transferasa (76%), que son marcadores útiles del proceso inflamatorio en el hígado. Los purés no alteraron el metabolismo de los lípidos y se observó una mejora notable en la morfología hepática. El orden de las dietas con los mejores efectos beneficiosos fue: dieta suplementada con puré de guayaba-zarzamora > guayaba-fresa, guayaba-maracuyá > guayaba-guanábana. El consumo de purés de frutas representa una alternativa terapéutica para los individuos diabéticos.

1. Introduction
Diabetes mellitus (DM) is a chronic and heterogeneous disease with hereditary predisposition that is influenced by various environmental factors. It is characterized by chronic hyperglycaemia caused by alterations in the metabolism of carbohydrates, fats and proteins, as a consequence of defects in the secretion or activity of insulin (BrownBrownlee et al., 2016).

According to the World Health Organization (WHO, 2016), there were 1.6 million deaths by DM in 2015 and it was estimated that DM will become the seventh cause of death...
in 2030. This disease affects adults and children, especially those who are overweight or obese.

The pathophysiology of DM involves a progressive deterioration in the integrity of pancreatic beta cells, responsible for insulin secretion in response to the increased glycemia. This increase leads to glucotoxicity, that causes alterations in the function of insulin receptors (BrownBrownlee et al., 2016). It has been suggested that oxidative stress plays a fundamental role in the pathogenesis of this disease because chronic hyperglycemia increases the production of reactive oxygen species (ROS), through several mechanisms; namely, glucose oxidation, protein glycosylation and free fatty acids oxidation (Wang, Tao, & Hai, 2012). An imbalance between the production of free radicals and the antioxidant defense status may cause damage to macromolecules, resulting in cellular dysfunction and injury (Vahid, Zand, Nosrat-Mirshekarlou, Najafi, & Hekmatdoost, 2015).

DM causes adipocyte dysfunction that secretes different substances to the circulation stream. These substances include hormones, cytokines, such as leptin, interleukin 6 (IL-6) interleukin-8 (IL-8), tumor necrosis factor alpha (TNF-α), and adiponectin, among others; all of them directly affect hepatocytes and skeletal muscle cells, favoring insulin resistance (Touskova et al., 2012). Insulin resistance is involved with deficiencies in the function of the main digestive organ: the liver. Cellular dysfunction of hepatocytes causes elevated serum concentrations of liver enzymes such as aspartate aminotransferase (AST), alanine aminotransaminase (ALT), Y-glutamyl transferase (GGT) and alkaline phosphatase (FA), which clinically translates into hepatic inflammation (Al-Fari, Al-Sawadi, & Alokal, 2010). These changes frequently cause diabetic liver in patients with increased risk of fibrosis and other immunological complications caused by loss of liver function as protein and glucose synthesis, bile excretion, nutrients storage and depletion of toxic substances, among others (Cabiati et al., 2013).

Good eating habits, physical activity and consuming foods that contain bioactive compounds such as flavonoids, carotenoids, dietary fiber among others, offer an excellent opportunity of intervention to mitigate the risk of DM (Wu, Zhang, Jiang, & Jiang, 2015). The effects of the bioactive compounds from fruits and vegetables have been studied, as well as aqueous extracts and/or isolated compounds and their ability to reduce metabolic risk markers (Azofeifa et al., 2016; Díaz-de-Cerio et al., 2017). However, the functional potential of processed foods containing a combination of fruits has rarely been studied. We investigated the effect of intake of fruit purees on plasma glucose, in hyperglycemic rats and we demonstrated that all purees decreased the plasma glucose in hyperglycemic rats (Pérez-Beltrán et al., 2017); therefore, derived from our first study and the fact that we did not induce DM in rats; the aim of this work was to evaluate the changes in plasma glucose and liver biomarkers in diabetic rats caused by the intake of fruit purees.

2. Materials and methods

2.1. Fruit purees

Four puree formulations: guava-strawberry (GSP), guava-blackberry (GBP), guava-soursop (GSSP) and guava-passion fruit (GPP) purees, were elaborated and donated by Purés y Derivados de Nayarit (PDN), Mexico. The nutritional composition and content of bioactive compounds of fruit purees were described in a previous publication (Pérez-Beltrán et al., 2017).

2.2. Experimental diets

Five diets were used. A commercial pedestrian maintenance diet for experimentation (Nutri cubo Purina®; Agribands Purina Mexico SA de CV., Mexico City, Mexico) was considered as the standard rodent diet (StD). The other four diets were prepared with StD (87%) and supplemented with a type of fruit puree (13%). The nutritional composition of diets was added as supplementary data (Table S1).

2.3. Animals and experimental design

The State Bioethics Committee of Nayarit, Mexico, approved the experimental protocol (No. CENB/03/2017) and also the protocols of the Institutional Animal Care and Use Committee according to Mexican standards (NOM-062-ZOO-1999, 2017) were followed. Animals were obtained from the Institute of Neurobiology of the UNAM (Queretaro, Mexico). Thirty-six adult female Wistar rats (245 ± 5 g) were used in this experiment. They were kept on a 12 h light/12 h darkness cycle at a temperature of 23 ± 1°C, with free access to food and water. Rats were placed in individual cages; after 1 week of acclimation, the animals were randomized into six groups of six rats each. They were weighed and assigned to the control group (healthy) or the experimental groups.

The healthy control group was fed with 15 g StD during all the experiment. To induce DM, the rats were injected with intraperitoneal injections of streptozotocin (STZ, Sigma-Aldrich, St. Louis, MO), one every week for 4 consecutive weeks after fasting overnight. The rats were injected with STZ at 65 mg/kg, dissolved in citrate buffer (pH 4.5). Once DM was induced, a glucose test was performed to verify the effect of the drug. After 8 h of fasting, tail vein blood samples were collected to determine blood glucose levels and basal insulin. All diabetic rats were divided in five groups. The groups were as follows:

- Group I: healthy control group fed with StD (H-StD)
- Group II: diabetic group fed with StD (DM-StD)
- Group III: diabetic group fed with StD + guava-strawberry puree (DM-StD + GSP)
- Group IV: diabetic group fed with StD + guava-blackberry puree (DM-StD + GBP)
- Group V: diabetic group fed with StD + guava-soursop fruit puree (DM-StD + GSSP)
- Group VI: diabetic group fed with StD + guava-passion fruit puree (DM-StD + GPP)

Over the dietary intervention period, the diets and water were administered orally ad libitum. The quantities of diet every day for 4 weeks were: 15 g StD to the H-StD and DM-StD groups and 13 g StD supplemented with 2 g of a different fruit puree to the other diabetic rats.

The food intake of the rats was recorded daily, and their body weights were monitored each week throughout the experiment. After 8 weeks, the animals were killed after 12 h of fasting to obtain blood and hepatic tissue. The euthanasia protocol was followed according to the Manual on the Use and Care of Experimental Animals (NOM-062-ZOO-1999, 2017).

The blood samples were obtained from in the inferior vena cava. Serum samples were prepared by centrifuging the blood samples (1.5 mL) at 4500 g for 5 min at 4°C, and...
were analyzed within 7 h, in order to comply with the quality control requirements of clinical biochemical tests (Ángel-Mejía & Ángel-Ramelli, 2006).

2.4. Plasma glucose, insulin, liver enzyme activity and other metabolites

The concentration of plasma glucose and insulin (basal and postprandial), liver enzyme activity (AST, ALT, GGT and ALP), as well as, glycosylated hemoglobin (HbA1c), urea, creatinine, uric acid, total proteins (TP), albumin and bilirubin (direct and total) were assessed according to the methods of the International Federation of Clinical Chemistry, using enzymatic colorimetric kits (Biosystems Reagents and Instruments, Barcelona, Spain) with the BioSystem Auto-analyzer (BTS-350, Barcelona, Spain). Postprandial glucose and insulin were determined in serum at time 0 (baseline) and at 2 h after oral administration of 3 mL of glucose solution (3 g/kg). The results are expressed as units per liter (U/L). The insulin content was measured in plasma using the sensitive rat insulin radioimmunoassay kit (Linco Research, Inc., St. Charles, MO).

2.5. Lipid profile

Total cholesterol (TC), triacylglycerols (TG), and high-density lipoprotein cholesterol (HDL-C) were determined in the serum samples using enzymatic colorimetric kits (Biosystems Reagents and Instruments, Barcelona, Spain) and a calibrated BioSystem Auto-analyzer (BTS-350, Barcelona, Spain). The low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) fractions were determined according to the Friedewald equations (Siri-Tarino & Krauss, 2016).

2.6. Histological analysis

The liver was fixed in 10% neutral-buffered formalin (pH 6.8) and embedded in paraffin with a Leica Biosystems TP1020 tissue processor (Nussloch, Germany). The tissues were cut in sections with a Leica RM2125RTS microtome (Nussloch, Germany) and stained with hematoxylin and eosin (Al Sayed et al., 2016). The sections were analyzed using a Leica DME1359 light microscope (Wetzlar, Germany) (40x magnification).

2.7. Statistical analysis

In vivo data are expressed as mean of three replicates ± standard error (SE). Data were analyzed using one-way analysis of variance (ANOVA, p < 0.05) and mean comparisons using the least significant difference test ($\alpha = 0.05$). All statistical analyses were performed using the statistical software STATISTICA (v.10 StatSoft, Tulsa, OK).

3. Results and discussion

3.1. Body weight

During 4 weeks post-DM induction, the body weight in DM groups decreased 41% (Figure 1). The loss of body weight in diabetic individuals could be associated with dehydration (polyuria), lipid catabolism and loss of muscle mass caused by the deficiency in protein synthesis (Nath, Ghosh, & Choudhury, 2017). However, after 8 weeks of feeding with StD and a fruit puree, all DM groups recovered their body weight with similar values at H-StD group. This recovery of body weight of the diabetic groups can be attributed to glycemic control (glucose homeostasis), because there is a relationship between the consumption of dietary fiber and an increase in the production or time of action of a peptide similar to glutathione 1 (GLP-1); thereby the increase in insulin secretion in a glucose-dependent manner,

![Figure 1](https://example.com/figure1.png)

Figure 1. Body weight of the experimental animals over induction (1–4 weeks) and over the dietary intervention period (5–8 weeks). Healthy rats fed with standard diet (H-StD), diabetic rats fed with standard diet (DM-StD) and diabetic rats fed with standard diet plus guava-strawberry puree (DM-StD+GSP), guava-blackberry puree (DM-StD+GBP), guava-soursop puree (DM-StD+GGP) or guava-passion fruit puree (DM-StD+GPP). The values represent means ± standard error. NS = not significant. Means statistically different *(p < 0.05), ** (p < 0.001).

Figura 1. Peso corporal de los animales de experimentación durante la inducción (1–4 semanas) y durante el periodo de intervención dietética (5–8 semanas). Ratas sanas alimentadas con dieta estándar (H-StD), ratas diabéticas alimentadas con dieta estándar (DM-StD) y ratas diabéticas alimentadas con dieta estándar más puré de guayaba-fresa (DM-StD+GSP), guava-blackberry puree (DM-StD+GBP), guava-soursop puree (DM-StD+GGP) o guava-passion fruit puree (DM-StD+GPP). Los valores son la media ± el error estándar. NS = no significativo. Medias estadísticamente diferentes *(p < 0.05), ** (p < 0.001).
the control in the secretion of glucagon promoting the use of blood glucose, as well as the absorption of other nutrients, contributed to the recovery of body weight of diabetic animals (Ding et al., 2016).

### 3.2. Plasma glucose, insulin and other metabolites

Table 1 shows the results of plasma glucose and insulin. After an 8-h fast, the glucose and insulin (basal) content in the DM-StD group show a significant difference (p<0.05) with respect to the H-StD group. A significant increase in glucose at the beginning (265.17 mg/dL) and at the end (291.33 mg/dL) of the intake of the glucose solution in the DM-StD group was observed, for this reason the percentage of HbA1c was 11.11% with respect to the healthy group (3.8%). In a diabetic individual, the levels of these biochemical markers are maintained during a period of 3 months (3.8%). In a diabetic individual, the levels of these biochemical markers are maintained during a period of 3 months (3.8%). In a diabetic individual, the levels of these biochemical markers are maintained during a period of 3 months (3.8%). In a diabetic individual, the levels of these biochemical markers are maintained during a period of 3 months (3.8%). In a diabetic individual, the levels of these biochemical markers are maintained during a period of 3 months (3.8%). In a diabetic individual, the levels of these biochemical markers are maintained during a period of 3 months (3.8%). In a diabetic individual, the levels of these biochemical markers are maintained during a period of 3 months (3.8%).

However, an increase of postprandial glucose and insulin (291.33 mg/dL and 18 µU/mL, respectively) in the DM-StD group was observed. STZ-induced diabetes is an accepted model of experimental diabetes that causes alterations similar to those observed in diabetic patients (Nath et al., 2017). STZ causes necrosis of pancreatic beta cells, reduces insulin synthesis and prevents glucose uptake by the recipient cells of liver, skeletal muscle, and adipose tissue. Given the lack of pancreatic function and that insulin secretion is greatly reduced, there is an increase of basal glucose and postprandial glucose in diabetic animals (Prasath & Subramanian, 2011). The increase of postprandial insulin in the DM-StD group can be prompted by insulin secretion by pancreatic beta cells that were not brought to apoptosis. However, this insulin may not be functional. Glucotoxicity generates conformational changes to insulin receptors, and alter their function (BrownBrownlee et al., 2016). This is probably the main reason why the DM-StD group did not show a reduction in glucose concentration at the beginning or end of the ingestion of the glucose solution.

On the other hand, in the DM groups fed StD plus a fruit puree (DM-StD+FP), the glucose (basal and postprandial) concentrations were significantly reduced (p<0.05). Meanwhile, basal and postprandial insulin activities were similar to the StD group. The GBP and GSP were more effective in glucose reduction. When control of plasma glucose occurred for several weeks, the percentage of Hb Glyc A1c was kept within the reference values (Bryce et al., 2017). We also observed that the percentage of Hb A1c in the DM-StD+FP group was reduced to ~47.9%.

There are diverse mechanisms by which bioactive compounds control the plasma glucose. Some reports have documented an effective glycemic reduction after consuming guava extracts rich in phenolic compounds such as chlorogenic acid, gallic acid, quercetin, myricetin and rutin (Díaz-de-Cerio et al., 2017; Liu, Wang, Hsieh, Lu, & Chiang, 2015). Modulation of cell signaling pathways by polyphenols in pancreatic cells, hepatocytes, adipocytes, and skeletal muscle cells are among the proposed mechanisms. Polyphenols may exert anti-diabetic effects by enhanced insulin production, reduced apoptosis or by increasing the proliferation of pancreatic cells. These mechanisms regulate glucose metabolism by interfering with its absorption or by increasing peripheral tissue glucose uptake through the insulin receptor-dependent or independent mechanisms; and also, through the modification of oxidative stress, inflammation or the energy status of the cell (Edirisinge & Burton-Freeman, 2016).

Polyphenols have been identified as effective food constituents that improve the sensitivity to insulin by inhibition of the serine protease dipetidyl peptide dipeptide IV (DP-IV). DP-IV is a membrane-associated peptidase that is distributed in various tissues and inactivates the insulinotropic peptides: the glucagon-like peptide 1 (GLP-1) and the glucose-dependent insulinotropic polypeptide (GIP). Both of these peptides are considered potent insulin secretion stimuli; thus, the inhibition of DP-IV leads to enhanced insulin secretion and sensitivity (Liu et al., 2009). Instead, under diabetic conditions, dietary fiber and/or the indigestible fraction of fruit purées may stimulate the production of short-chain fatty acids, which enhance glucose oxidation, reduce the release of free fatty acids and improve insulin sensitivity. Also, synthesis of the glucagon like-peptide 1 (GLP-1) can be induced, which is responsible for delayed gastric emptying, increased insulin-dependent cellular uptake of glucose.

### Table 1. Biochemical markers for healthy animals fed with standard diet (H-StD), diabetic animals fed with standard diet (DM-StD), and diabetic animals fed with standard diet plus guava-strawberry puree (DM-StD+GSP), guava-blackberry puree (DM-StD+GBP), guava-soursop fruit puree (DM-StD+GSSP) or guava-passion fruit puree (DM-StD+GPP).

| Parameters                      | H-StD     | DM-StD   | DM-StD+GSP | DM-StD+GBP | DM-StD+GSSP | DM-StD+GPP |
|--------------------------------|-----------|----------|------------|------------|-------------|------------|
| Basal glucose                  | 77.83 ± 2.22a | 265.17 ± 3.85a | 108.17 ± 2.67a | 87.17 ± 2.67a | 108.67 ± 2.23a | 97.20 ± 3.45a |
| Postprandial glucose           | 82.83 ± 2.30a | 291.33 ± 2.37a | 104.33 ± 3.25bc | 91.67 ± 2.89bc | 99.00 ± 2.6a | 122.60 ± 8.53c |
| Basal insulin                  | 11.83 ± 1.10a | 62.6 ± 0.47 | 10.83 ± 0.42 | 12.44 ± 1.33 | 11.88 ± 2.13 | 11.09 ± 0.41b |
| Postprandial Insulin           | 8.07 ± 0.24a | 18.06 ± 0.48a | 8.35 ± 1.07 | 6.63 ± 0.62 | 9.41 ± 0.62a | 7.22 ± 0.70a |
| Glycosylated hemoglobin (HbA1c)| 3.84 ± 0.16a | 11.11 ± 1.31a | 5.40 ± 0.30a | 5.08 ± 0.46 | 6.78 ± 0.81b | 5.16 ± 0.29b |
| Urea                           | 55.00 ± 4.57 | 48.33 ± 4.07ab | 45.83 ± 3.18ab | 49.00 ± 1.50ab | 37.50 ± 2.18b | 54.40 ± 4.73a |
| Uric acid                      | 2.67 ± 0.37a | 1.83 ± 0.31a | 2.88 ± 0.46a | 3.18 ± 0.49a | 1.58 ± 0.37a | 2.70 ± 0.37a |
| Creatinine                     | 0.65 ± 0.02a | 0.98 ± 0.08a | 0.82 ± 0.08a | 0.83 ± 0.09b | 0.77 ± 0.05a | 0.80 ± 0.15a |

Units of Glucose, Urea, Creatinine and uric acid = mg/dL; Insulin = µU/mL; HbA1c = % Hemoglobin. The values are means ± standard error. Different letters in the same file, represent significant statistical differences (p < 0.05) between treatments.
inhibited glucagon secretion, stimulated insulin secretion and reduced hepatic glucose production, which collectively may act to reduce the requirement for insulin (Galisteo, Duarte, & Zarzuelo, 2008).

The experimental fruit purees had a high content of an indigestible fraction, namely polyphenols, carotenoids and vitamin C; although the GBP and GSP had the highest content of polyphenols (Pérez-Beltrán et al., 2017), coinciding with the greatest glucose reduction in the DM groups fed with StD plus GBP or GSP. Anthocyanins isolated from berries inhibit the affinity of the facilitated glucose transporter 2 (GLUT2), which is the dominant apical intestinal sugar transporter when the intestinal glucose concentrations are high and the affinity for the sodium dependent glucose transporter 1 (SGLT1) after the dietary ingestion (Forbes et al., 2016). Anthocyanins can cross from the apical side into cells, and/or to the basolateral side, and consequently inhibit the GLUT2 efflux on the basolateral side of the cells. This decreases the capillary and venous plasma glucose, the serum insulin concentrations as well as the glucagon-like peptide 1 (GLP-1) response, therefore improving the glycemic profile (Torronen et al., 2012). These compounds seem to regulate the redox status and the antioxidant defense system, as it was reported by Pérez-Beltrán et al. (2017). Consumption of fruit purees decreased plasma glucose in hyperglycemic rats, reducing glucotoxicity.

Table 1 shows the values of urea, creatinine and uric acid of healthy animals and the diabetic groups with and without the addition of fruit purees. Significant changes (p > 0.05) on these metabolites were not found. These metabolites are useful for the analysis of kidney function in chronic diabetic patients. This means that the rat kidneys satisfied the functions of excretion and reabsorption adequately (de Oliveira et al., 2016).

### 3.3. Liver enzyme activity

Figure 2 shows the activities of AST, ALT, GGT and PA in the diabetic model. Serum AST activity (Figure 2(a)) was not different (p > 0.05) in all evaluated groups. STZ is a drug metabolized by the liver and is specific to produce apoptosis in pancreatic beta cells (Nath et al., 2017). AST is found in the cytoplasm and mitochondria of the hepatocyte and an increase in its activity indicates apoptosis or cell lysis (Fishbein, Miner, Mogren, & Chalekson, 2003). According to these data, it is possible to assume that the fractionated doses of STZ did not cause significant damage in hepatocytes.

Figure 1b and c show serum ALT and ALP activities. Serum ALT activity (Figure 2(b)) was higher (62 U/L) in the DM-StD group than the H-StD group (38.17 U/L) and DM-StD+FP groups (24.60–36.0 U/L, p > 0.05). The same mode, serum ALP activity was higher in the DM-StD group (152.50 U/L) than the H-StD group (49.33 U/L) or DM-StD+FP groups (46.60–80.33 U, p > 0.05). Fruit purees decreased the ALT and ALP activities in 42–60% and 47–69%, respectively. The increase of serum ALT and ALP activities in the DM groups reflect a clinical state of inflammation of the liver, caused by free radicals, as well as the pro-inflammatory factors secreted by adipose tissue such as interleukins (IL-6, IL-10) and tumor necrosis factor (TNF-α) (Wang, 2015). It specifically indicates...
damage to hepatic sinusoids, a structure that involves the exchange of metabolites and/or nutrients between the blood and hepatocytes (Lasram et al., 2014). A poor control in the metabolic regulation of endogenous antioxidant enzymes as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) cause stress in the mitochondrial and peroxisomal levels that produce an increase the activity of liver enzymes (Corey & Kaplan, 2014).

Consumption of fruit purees had a significant effect on the decrease of ALT and ALP activities, probably triggered by their content of bioactive compounds that act as antioxidants. Pérez-Beltrán et al. (2017) reported that the four purees contained significant quantities of polyphenols (gallic acid, quercetin-3-o-glucoside and quercetin) and vitamin C; also the GBP and GSP contained cyanidine-3-glucoside and pelargonidin-3-glucoside, respectively. These bioactive compounds have potential effects as chelators, free radical scavengers and inhibitors of the mechanisms involved in inflammation and cellular apoptosis (Fumagalli et al., 2016). The importance of the consumption of flavonoids such as quercetins, catechins and isoflavones in the control of inflammatory processes is mentioned because these compounds suppress the secretory pathway of pro-inflammatory factors that directly lead to inflammation in liver (Amaya-Cruz et al., 2015). Also, it was found that the gallic acid increased the expression of genes that synthesize antioxidant enzymes such as SOD and CAT; modulated the expression of molecules that cause inflammation and also induced the expression of genes involved with cell proliferation, all contributing to a hepatoprotective effect, if the liver regenerated ALT activity is low (de Oliveira et al., 2016). Our results coincide with reports on diabetic rats fed with a diet supplemented with strawberries, in which the decrease of markers of inflammation was observed (Giampietri et al., 2016).

Serum GGT activity was 5.5 U/L for the H-StD group and 7.0 U/L for the DM-StD group (Figure 2(d)). Clinically, an increase of this enzyme indicates inflammation in the liver (bile ducts). A chronic excess of glucose exacerbates the damage in the pancreas causing glucotoxicity involving the activation of apoptosis with the concomitant increase in the secretion of IL-6 and TNF-α, which in turn cause oxidative stress in hepatic structures (Giampietri et al., 2016). Therefore, an increase in its concentration indicates biliary obstruction as it probably occurred in the DM-StD group. However, a beneficial anti-inflammatory outcome was produced by fruit purees, since there was a decrease of 76% of serum GGT activity in the DM-StD+FP groups with respect to the DM-StD group and 70% in the H-StD group.

The hepatoprotective effect of bioactive compounds through different mechanisms of action has been proposed. However, the anti-inflammatory effect of bioactive compounds, primarily antioxidants, which is related to the decrease in the concentration of hepatic enzymes, suggests that these compounds, once absorbed and circulating in the bloodstream, inhibit the cytotoxic activity of ROS (generated by glucotoxicity) and the corresponding inflammation markers (Fumagalli et al., 2016; Leelarungrayub, Laskin, Bloomer, & Pinkaew, 2016).

The total protein, and in particular albumin are involved in the transport of nutrients, participate in the osmotic balance and significantly contribute to the mechanisms involved in the immunity. An increase of these proteins favors the transport of different nutritional elements and bioactive compounds to different target organs, but if the serum protein concentration is low, it implies a failure of liver function (Jin et al., 2016). Total protein and albumin of the serum from the H-StD group were 9.33 and 4.15 mg/dL, respectively (Figure 3(a) and (b)); however, in the DM-StD group the concentration of these metabolites decreased (7.33 and 2.47 mg/dL, respectively). Nonetheless, a significant increase (25–46% and 54–62%, respectively) in the content of both parameters was observed in the DM-StD+FP groups, suggesting an improvement in the immune system in response to the metabolic disorders of the diabetic pathology (Jin et al., 2016), although it was clear that GSP and GBP had the highest effect (p < 0.05).

Figure 3c,d show that the DM-StD group had the highest serum values of total bilirubin (0.75 mg/dL) and direct bilirubin (0.98 mg/dL); this increase suggested the obstruction of the bile ducts caused by liver inflammation (Masubuchi et al., 2016). Fruit purees favored the metabolism in DM groups, because judged by a decrease in the concentration of direct bilirubin with a similar range (0.24–0.52 mg/dL) than the H-StD group (0.37 mg/dL), with values of total bilirubin (0.33–0.48 mg/dL) slightly greater than the H-StD group (0.17 mg/dL) but lower than the DM-StD group. The typical concentrations of total and direct bilirubin reflect a balance between hepatobiliary production and excretion; however; an increase of total bilirubin indicates the existence of intra or extra hepatic obstructive processes, while high concentrations of direct bilirubin suggest the narrowing of bile ducts causing inflammation. Both processes have been associated with pathologies such as obesity, diabetes mellitus type II and metabolic syndrome, among others (Lee et al., 2016). Consumption of antioxidant compounds and dietary fiber through ingestion of fruit purees are related to the decrease of tissue damage in rats caused by free radicals’ neutralization; therefore, if these radicals decrease, it could also decrease the concentration of bilirubin because it implies a repaired tissue (Amaya-Cruz et al., 2015).

### 3.4. Lipid profile

The contents of TC, TG, HDL-C, VLDL-C and LDL-C are shown in Table 2. In almost all DM rats, there were no significant differences (p < 0.05) in the TC content, except in the case of the DM+GSP group, which exhibited an increase of 49% in TC; however, the TC value is within the reference values indicated in human metabolism (Touskova et al., 2012). Also, the HDL-C levels remained stable in all DM groups during the treatments, without significant differences (p > 0.05), which was consistent with other reports (Pérez-Beltrán et al., 2017).

In the H-StD group we found 51 mg/dL of TG, while in the DM groups with and without the addition of fruit purees to the diet, we recorded values from 96 to 111 mg/dL without significant differences (p > 0.05). A decrease of TG in hyperglycemic rats was reported when they were fed fruit purees (Pérez-Beltrán et al., 2017). The difference with this experiment is that the animals were induced with DM, so that insulin resistance was probably developed that triggered the triacylglycerol movement in the adipose tissue to produce energy. This mobilization may have caused alterations in the metabolism of lipoproteins (Bavia, Cogliati, Dettoni, Ferreira Alves, & Isaac, 2016); therefore, it may trigger a significant increase in the concentration of LDL-C and VLDL-C (see Table 2) in contrast with the H-StD group. DM
caused changes in the anti-lipolytic effect of insulin, increased the lipolysis cycle and induced hypertriglyceridemia through the production of LDL-C and VLDL-C (Chen et al., 2012). Thus, consumption of GSP, GBP, GSSP or GPP fruit purees did not alter lipid metabolism in diabetic rats.

3.5. Histological analysis

The normocytic and normochromic hepatocytes of the H-StD group with normal chroma and uniform size are shown in the microphotography of Figure 4a, in which the physiomorphology of a healthy individual can be observed. Figure 4b depicts the histological details of the DM-StD group. Dysmorphic hepatocytes can be observed, with enlarged nucleus and cytoplasmic space, as well as delayed regeneration of hepatocytes with respect to the H-StD group (Figure 4a), which indicated an inflammatory process. Glucotoxicity produces insulin resistance, which in turn generates ROS that damage the liver membrane structure, causing an inflammation of the sinusoidal spaces (Brownlee et al., 2016).

In the Figure 4c-f, changes of the hepatic morphology in response to consumption of fruit purees in the diet of DM rats can be observed. The histological analysis showed a marked improvement in these groups, homogeneous hepatocyte cells, normocytic and normochromic endothelial cells and normal hepatic sinusoids. Also, there was a decrease in the number of intracellular vesicles, which represent a reduction in lipid deposits (Giampieri et al., 2016). In this sense, a possible balance in glucose metabolism could be observed, since the cytoplasm and the
intracellular material are noted with greater intensity. The potential effect by the addition of fruit purees in the diet was confirmed, considering that the high content of dietary fiber and bioactive compounds together reduced plasma glucose, the oxidative stress and inhibited the ROS toxicity (Azofeifa et al., 2016; Pérez-Beltrán et al., 2017).

Vahid et al. (2015) reported that for the regeneration of new liver cells, gene transcription is necessary, which occurs when polyphenols prevent the action of the substrate by deacetylases by competitive inhibition, promoting the relaxation of histones and as a result of gene transcription, inducing cell division (cell proliferation). The same authors reported the effect of various compounds such as resveratrol, epigallocatechin-3-gallate (EGCG), quercetin, ellagitannins, organosulfonates at the DNA level, which act in a directly proportional relationship of the concentration of histone deacetylase enzymes with respect to the population of hepatocytes. García-Niño and Zazueta (2015) mentioned that the metabolic system converts or biotransforms antioxidants that are absorbed by the enterocytes, exposing their functional groups or joining them to their structure. They enter the bloodstream to be transported to the liver where they are again metabolized and thus they generate a hepatoprotective effect.

4. Conclusions

Additional consumption of four fruit purees in the diet improved body weight in DM rats and did not alter their lipid metabolism. Also, the prolonged consumption of fruit purees controlled the secretion of insulin, decreased plasma glucose concentrations and had an anti-inflammatory effect on the liver, with a significantly decrease in ALT, ALP and GGT activities. The reduction in bilirubin suggested a strong detoxification. Consumption of fruit purees is proposed as a healthy choice as anti-diabetic and anti-inflammatory foods.

Disclosure statement

No potential conflict of interest was reported by the authors.

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