Rev Bras Farmacogn 24(2014): 341-347

Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.journals.elsevier.com/revista-brasileira-de-farmacognosia

Original article

Phytochemical screening and hypoglycemic activity of *Carica papaya* leaf in streptozotocin-induced diabetic rats

Isela E. Juárez-Rojop[a], *Carlos A.Tovilla-Zárate*[b], *Dora E. Aguilar-Domínguez*[a], *Luis F. Roa-de la Fuente*[c], *Carlos E. Lobato-García*[c], *Jorge L. Blé-Castillo*[a], *Leonor López-Meraz*[d], *Juan C. Díaz-Zagoya*[e], *Deysi Y. Bermúdez-Ocaña*[b,*]

[a]*División Académica de Ciencias de la Salud, Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco, Mexico*
[b]*División Académica Multidisciplinaria de Comalcalco, Universidad Juárez Autónoma de Tabasco, Comalcalco, Tabasco, Mexico*
[c]*División Académica de Ciencias Básicas, Universidad Juárez Autónoma de Tabasco, Cunduacán, Tabasco, Mexico*
[d]*Universidad Veracruzana, Xalapa, Veracruz, Mexico*
[e]*Departamento de Bioquímica, UNAM, México, D.F., Mexico*

ARTICLE INFO

Article history:
Received 16 December 2013
Accepted 2 April 2014

Keywords:
Carica papaya
Diabetes
Streptozotocin
Steroids
Tannins
Quinones
Alkaloids

A B S T R A C T

The extraction of plant constituents is essential to isolate biologically active compounds, aimed to understand their role on the treatment of diabetes. This study was designed to explore the preliminary phytochemical and physicochemical analysis of *Carica papaya* L., Caricaceae, leaf, and further evaluation of its hypoglycemic effect on diabetic rats. *C. papaya* leaves were extracted using chloroform, *n*-hexane or ethanol. For each extract a phytochemical screening was performed. The tests were conducted in triplicate and the qualitative and quantitative determination of the various metabolites was done using analytical standards proposed by Mexican Herbal Pharmacopoeia. The chloroform extract, containing steroids and quinones as major components, was chosen to study *C. papaya* biological effects. The chloroform extract was evaporated to dryness, and doses 0, 31, 62, 125 mg/kg were orally administered in 300 µl polyethylene glycol to diabetic rats; and 0 and 62 mg/kg to non-diabetic rats. After a 20-day treatment with the chloroform extract, the animals were sacrificed and blood was obtained for biochemical studies. The main effect observed was a decrease in serum glucose, triglycerides and transaminases in diabetic rats after the administration of *C. papaya* chloroform extract. These results confirm the potential beneficial action of *C. papaya* to treat the symptoms of diabetic patients.

Introduction

Diabetes mellitus is possibly the world's largest growing metabolic disorder. Global prevalence of diabetes has dramatically continued to increase. The difficulty of managing hyperglycemia in diabetes is the most important factor in reducing the risks associated with diabetes and its complications (Polonsky, 2012). Both fasting and postprandial glucose regulation are critical to achieve a long-term proper control in diabetic patients. The number of diabetic patients is rapidly increasing, and in consequence the control of their complications is a challenge. In this regard, medicinal plant

* *Corresponding author.*
E-mail: deysi.bermudez@ujat.mx (D.Y. Bermúdez-Ocaña).
0102-695X/$ - see front matter © 2014 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. All rights reserved.
http://dx.doi.org/ 10.1016/j.bjp.2014.07.012

extracts have an ancient background in this issue and modern medicine can gain valuable benefits from them. Phytochemicals and their derived products have been an extraordinary source of compounds with therapeutic and drug development potential (De D et al., 2012). These molecules are novel and complex structures that can be used in their original form, or can serve as lead molecules to develop derivatives with higher specificity and fewer side effects (Koehn and Carter, 2005). The World Health Organization has been particularly attentive to the potential offered by herbal medicine, the main subfield of traditional medicine practiced in different countries (WHO, 2012). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes. Mexico has an extensive and diverse medicinal flora whose properties are part of the ancestral knowledge. Currently, natural compounds used as hypoglycemic agents have a strong impact on diabetic patients.

Carica papaya L., is an herbaceous plant with prominent leaves (20-60 cm long), and is a member of the Caricaceae family, indigenous to the tropical region of Mexico, Central America and northern South America. *C. papaya* is distributed throughout the tropics and subtropics where it is extensively cultivated. The characterized metabolites from the plant are chitinase, glutaminyl cyclase and cysteine endopeptidases of class-II and III from Carica latex (Azarkan et al., 2006); linalool in fruit pulp, and alkaloids such as carpaine, pseudocarpaine, dehydrocarpaine I and II (Lim, 2012); and kaempferol and quercetin (Miean and Mohamed, 2001) in the leaves.

On the other hand, there are reports that describe the therapeutic effect of *C. papaya* leaf on dengue and malaria (Ahmad et al. 2011) and as anti-inflammatory (Owoyele et al., 2008). Other reports suggest that a fermented papaya preparation significantly reduces plasma glucose levels in healthy subjects and in patients with type 2 diabetes (Danese et al., 2006). The hypoglycemic activities of *Carica papaya* have been previously described for its fruit and leaves (Aruoma et al., 2010), nevertheless, the available information regarding the leaves is incomplete (Sasidharan et al., 2011). The present study was designed to perform phytochemical and physicochemical analyses of *Carica papaya* leaves, and to evaluate its hypoglycemic effect in diabetic rats.

Materials and methods

Plant material

Leaves from *Carica papaya* L., Caricaceae, were collected from June to September 2010 from Cintalapa, in the state of Chiapas, Mexico. The plant was authenticated at the Academic Division of Biological Sciences (DACB, acronym in Spanish) in the Juarez Autonomous University of Tabasco (UJAT, initials in Spanish) as *Carica papaya*. A voucher specimen was deposited in the herbarium (No. 32307) of this institution in Tabasco, Mexico.

Chemical products

Streptozotocin (STZ) was purchased from Sigma (St Louis, MO, USA). Insulin (Humulin* N) was obtained from Sanofi Aventis. All other chemicals of analytical grade were obtained from Merck. Kits for different enzyme assays were purchased from Biosystems S.A., Mexico.

Assays

Chemical analysis and quantitative assays of alkaloids, tannins, steroids, quinones and flavonoids content in C. papaya extract

Preliminary qualitative tests were carried out to determine the metabolites present in greater proportion in the leaf of *C. papaya*. Alkaloids, flavonoids, saponins, tannins, steroids and/or terpenes (triterpenoids), and quinones were identified. A total of 30 g of dried and ground *C. papaya* leaves were placed in a 250 ml round-bottom flask, where they were macerated and extracted with hexane, chloroform and ethanol. All procedures were developed at room temperature. The extracts were used for the subsequent qualitative analysis of metabolites. Once the main secondary metabolites were determined, a quantitative assay was developed using spectroscopic techniques as described by Mexican Herbal Pharmacopoeia (FHEUM, 2001). Each assay on the hexanic, ethanolic and chloroform extracts was performed in triplicate; 10 g of dried and ground leaves of *C. papaya* were exhaustively extracted with the corresponding solvent employing a Soxhlet system. After the extraction the solvent was removed under vacuum. Steroids were quantified by the modified Lieberman-Burchard reaction (Robin, 1945) at an absorbance of 550 nm. A cholesterol standard curve (2-8 mm) was made to report the concentration of steroids (Barreto, 2005). The quantification of tannins was performed by the reaction with ferric citrate, and then absorbance was read at 525 nm. A tannic acid standard curve (0.1-0.5 mg/ml) was prepared to report the concentration of tannins (ISO, 1988). Alkaloids were quantified by the reaction with bromocresol green read at 470 nm; a calibration curve with atropine (0.1-4 mg/ml) was constructed to report the alkaloid concentration (Fazel et al., 2008). For the quantification of quinones, the reaction with ferric chloride was employed and the absorbance was measured at 390 nm. A standard curve was prepared with a solution of 8-hydroxyquinone (0.010 to 0.06 mg/ml) to evaluate the concentration of quinones (Pochapski et al., 2011).

Preparation of C. papaya leaf chloroform extract

The leaves of *C. papaya* were washed with tap water and cut into small slices. The slices were pulverized after being air-dryed. Dry samples (100 g) were placed in the Soxhlet system, where the extraction was conducted for 8 h with 500 ml of chloroform. Afterwards, the solvent was evaporated under vacuum until the extract was completely dry, and was preserved at -20°C. The chloroform extract of *C. papaya* was used to a final concentration of 1 mg/ml.

Animals

Experiments were performed on adult male Wistar rats (body weight range: 250-300 g), 10 to 11 weeks of age. Animals were

housed and maintained at 22ºC under a 12:12 light/dark cycle, with free access to food and water. Experiments were carried out during the normal light/dark cycle and always started at the same hour (10 am). Efforts were made to minimize animal suffering and to reduce the number of animals used. All experiments complied with the Ethical Guidelines for the Use of Animals in Research; the study was approved by the local Internal Committee for the Care and Use of Laboratory Animals (003-10/CICUAL/DACS) of the Academic Division of Health Sciences (DACS, initials in Spanish), UJAT.

Induction of diabetes

Experimental diabetes (Courteix et al., 1994) was induced following an overnight fast by a single intraperitoneal injection of 60 mg/kg STZ (Sigma, St. Louis, MO, USA) freshly dissolved in distilled water. Control animals received 0.9% sterile saline. Hyperglycemia was confirmed four days after injection by measuring the tail vein blood glucose level with an Accu-Check Sensor Comfort glucometer (Roche, Mexico City). Only the animals with fasting blood glucose levels ≥ 250 mg/dl were included in the study. No rat was rejected in this study lot.

Study design

In order to determine the hypoglycemic effect of *C. papaya* leaves in diabetic rats, oral doses of *C. papaya* chloroform extract (31, 62 and 125 mg/kg) were administered via oral in 300 µl of polyethylene glycol. In this study we chose *C. papaya* leaves due to their traditional use by diabetic patients in the region of Cintalapa, Chiapas, Mexico. These doses were selected based on a previous study employing different amounts of aqueous extract of *C. papaya* leaves (Juárez et al., 2012). Experimental non-diabetic rats also received similar doses of *C. papaya* leaf chloroform extract. Insulin-treated diabetic rats (5 U/kg insulin, *i.p.*) served as positive control (Gupta et al., 2004). For each group of eight rats, the necessary biochemical determinations were carried out to identify the pharmacological effects of *C. papaya* leaves. For all animal groups, the body weight was measured at baseline and every week after. Following the 20 days of treatment, 12 h after food withdrawal, rats were sacrificed by decapitation.

Biochemical parameters

Blood was collected and serum was immediately frozen and stored at -70°C until biochemical determinations were performed. The serum levels of glucose, cholesterol, triacylglycerides, high-density lipoprotein-cholesterol (HDL-C), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were analyzed using a Clinical Chemistry System from Random Access Diagnostics. Plasma insulin concentrations were determined by an enzymatic immunoassay method (Bertin-pharma rat insulin ELISA).

Statistical analysis

All data are expressed as mean ± S.E.M. One-way analysis of variance (ANOVA) was performed followed by Tukey's test to compare the differences between treatments. Differences were considered statistically significant for $p < 0.05$.

Results

Chemical constituents of the extracts of C. papaya leaf.

Phytochemical analysis showed the presence of alkaloids and tannins in the ethanolic extract; and steroid and quinones in the three extracts. The amount of metabolites assayed is shown on Table 1. The highest concentrations of tannins ($4.85 \pm 10^{-3} \pm 1.76 \pm 10^{-4}$ M; 0.824%) and steroids ($5.98 \pm 10^{-3} \pm 2.20 \pm 10^{-3}$ M; 0.231%) were found in the ethanol extract of *C. papaya* leaf. Moreover, alkaloids ($1.04 \pm 10^{-4} \pm 1.78 \pm 10^{-6}$ M; 7.53 ± 10^{-4}%) and quinones ($2.20 \pm 10^{-5} \pm 5.81 \pm 10^{-7}$ M; 1.53 ± 10^{-4}%) were also identified. The *C. papaya* hexane extract contained both steroids ($9.32 \pm 10^{-3} \pm 1.45 \pm 10^{-4}$ M; 0.360%) and quinones ($1.27 \pm 10^{-5} \pm 4.80 \pm 10^{-7}$ M; 8.87 ± 10^{-5}%); whereas in the chloroform extract, the steroid concentration was notable ($4.34 \pm 10^{-2} \pm 5.25 \pm 10^{-3}$ M; 1.678%). As can be seen from the values, the chloroform extract showed the highest levels for both steroids and quinones. For this reason, such extract was used for the pharmacological tests.

Table 1
Alkaloids, tannins, steroids, quinones and flavonoid content in *C. papaya* extracts.

Chemical constituents	Extract (mean ± S.E.M; %)		
	Ethanol	*n*-Hexane	Chloroform
Alkaloids	$1.04 \pm 10^{-4} \pm 1.78 \pm 10^{-6}$; ($7.53 \pm 10^{-4}$)		
Steroids	$5.98 \pm 10^{-3} \pm 2.20 \pm 10^{-3}$; (0.231)	$9.32 \pm 10^{-3} \pm 1.45 \pm 10^{-4}$; (0.360)	$4.34 \pm 10^{-2} \pm 5.25 \pm 10^{-3}$; (1.678)
Quinones	$2.20 \pm 10^{-5} \pm 5.81 \pm 10^{-7}$; ($1.53 \pm 10^{-4}$)	$1.27 \pm 10^{-5} \pm 4.80 \pm 10^{-7}$; ($8.87 \pm 10^{-5}$)	$9.88 \pm 10^{-6} \pm 3.52 \pm 10^{-7}$; ($6.87 \pm 10^{-5}$)
Tannins	$4.85 \pm 10^{-3} \pm 1.76 \pm 10^{-4}$; (0.824)		

Data are expressed as molar (M) concentration ± SEM and as (percentage).

Effect of C. papaya leaf chloroform extract on body weight and blood glucose of STZ-induced diabetic rats

The STZ single-dose effect to induce type 1 diabetes in rats was confirmed by their significant weight loss and hyperglycemia as compared to non-diabetic animals ($p < 0.05$) (Fig. 1). The body weight was recorded every week; final data are shown in Fig. 1. There was a significant decrease in the area under the curve (AUC) of body weight of diabetic rats (3173 ± 120 g) compared with non-diabetic animals (3988 ± 110 g). The *C. papaya* chloroform extract (31 and 62 mg/kg) caused a minor reduction in body weight of diabetic rats ($p < 0.05$) than that displayed by control diabetic rats. In addition, this extract had a similar effect on the body weight AUC in diabetic rats receiving insulin (3639 ± 98 g). An interesting fact is that oral administration of *C. papaya* chloroform extract (31, 62 mg/kg) induced significant decreases of the glucose AUC of diabetic rats ($p < 0.05$), similarly to that observed in the group receiving insulin; both cases compared with the diabetic control group (not showed).

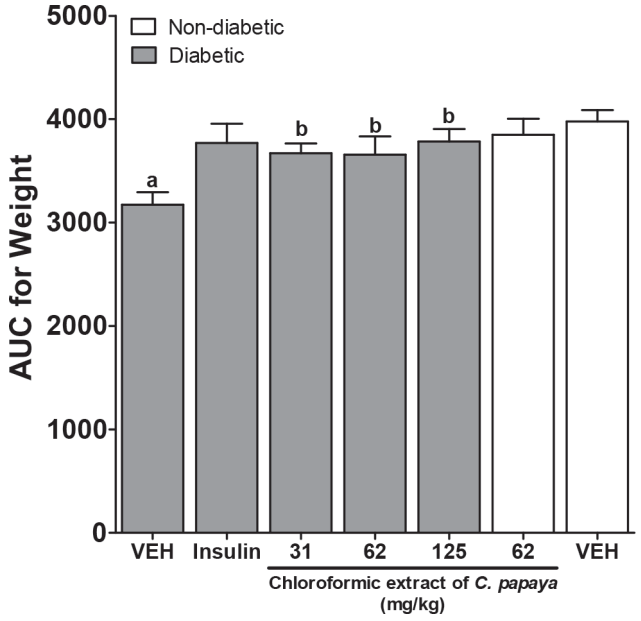

Figure 1 – Effects of *Carica papaya* leaf chloroform extract on the body weight of STZ-induced diabetic rats. Area under the curve of body weight obtained after a 20-day treatment with chloroform extract of *C. papaya* in diabetic rats. Results are presented as mean ± S.E.M.; number of rats per groups n = 8; $p < 0.05$, [a]compared to non-diabetic rats; bcompared to control diabetic rats. VEH, vehicle.

Biochemical parameters

Fasting blood glucose levels were highly elevated in diabetic animals ($p < 0.05$). After treatment with *C. papaya* chloroform extract (31-125 mg/kg), the fasting blood glucose level of diabetic rats was decreased compared with non-treated animals (Table 2). Administration of insulin to STZ-diabetic rats produced a significant reduction in fasting blood glucose level ($p < 0.05$) (Table 2). Serum cholesterol (Chol) levels in diabetic rats showed a significant elevation, in comparison to the values detected in non-diabetic control rats (88 ± 4.43 vs. 75.67 ± 3.28 mg/dl) (Table 2). Serum triacylglycerol levels were also elevated in diabetic rats when compared to non-diabetic animals (180.5 ± 12.5 and 84.67 ± 10.17 mg/dl, respectively) (Table 2). A 20-day administration of *C. papaya* leaf extract to diabetic rats did not change serum cholesterol levels, whereas serum triacylglycerol (TAG) levels decreased significantly in *C. papaya*-treated rats compared with diabetic control animals ($p < 0.05$). High-density lipoprotein cholesterol (HDL-C) levels did not change with the administration of *C. papaya* leaf extract to diabetic rats in comparison to diabetic control and non-diabetic control rats (Table 2). The insulin injection to STZ-diabetic rats produced a significant decline in serum Chol, TAG and HDL-C ($p < 0.05$) compared with diabetic controls.

The serum activities of AST and ALT, biomarkers of liver toxicity, were elevated in STZ-induced diabetic rats (395.8 ± 28.3, 238.8 ± 22.5 U/l, respectively) when compared with non-diabetic controls (249 ± 11.8, 56.3 ± 5.38 U/l, respectively) (Table 2). The treatment of diabetic rats with 31 and 62 mg/kg of *C. papaya* leaf extract significantly reduced the activities of these biomarkers with respect to control diabetic rats ($p < 0.05$), but treatment with 125 mg/kg did not reduce the activity of these serum enzymes in diabetic rats (Table 2). Administration of insulin to diabetic rats showed a significant decrease ($p < 0.05$) in serum transaminases as compared to STZ-diabetic rats (Table 2).

Determination of basal plasma insulin

The fasting plasma insulin levels were dramatically reduced in all groups of STZ-treated rats with or without *C. papaya* leaf extract (0.58 ± 0.09 ng/ml), when compared with non-diabetic control rats (1.06 ± 0.12 ng/ml) ($p < 0.001$) and diabetic rats treated with insulin ($p < 0.001$). Conversely, blood insulin levels were significantly increased in non-diabetic rats receiving 62 mg/kg of *C. papaya* leaf extract ($p < 0.05$) (Table 2).

Discussion

Currently, herbal products are being used as a source in medicine. The medicinal properties of plants have been part of ancient knowledge, and modern medicine benefits from them. In this sense, phytochemicals and their derivatives have been an extraordinary source of lead compounds for therapeutics and drug development.

Chemical constituents of the extracts of C. papaya leaf

In reference to the phytochemical analysis of *Carica papaya* L., Caricaceae, leaf extract, the presence of flavonoids, tannins, alkaloids and organic acids in a methanolic extract has been previously documented (Khuzhaev and Aripova, 2000; Head et al., 1957). According to Canini (2007), the main compounds contained by *C. papaya* leaves are phenolic acids, as well as trace amounts of chlorogenic acid, compared to flavonoids and coumarin compounds. In their study, the authors suggested that the presence of such phenolic and coumarin compounds

Table 2
Effects of *C. papaya* leaf chloroform extract on biochemical parameters of STZ-induced diabetic rats.

C. papaya mg/kg	Glucose (mg/dl)	Chol (mg/dl)	TAG (mg/dl)	HDL-C (mg/dl)	Ins (ng/ml)	AST (U/l)	ALT (U/l)
Diabetic							
control	344.5 ± 95.5[a]	88.0 ± 4.4[a]	180.5 ± 12.5[a]	33.9 ± 2.3	0.58 ± 0.09[a]	395.8 ± 28.3[a]	238.8 ± 22.5[a]
INS	94.6 ± 1.2[b]	57.1 ± 10.5[b]	50.8 ± 1.7 [b]	25.0 ± 6.2[b]	1.54 ± 0.25[b]	266.4 ± 24.2[b]	165.7 ± 15.9[b]
31	122.2 ± 36.1[b]	83.2 ± 7.6	94.3 ± 18.9[b]	33.4 ± 2.7	0.38 ± 0.06[a]	160.6 ± 25.5[b]	119.3 ± 18.1[b]
62	113.8 ± 25.1[b]	87.3 ± 8.7	68.5 ± 15.0[b]	35.3 ± 2.7	0.32 ± 0.09[a]	166.8 ± 7.1[b]	116.8 ± 25.1[b]
125	268.4 ± 82.6	84.7 ± 10.5	99.0 ± 12.2[b]	34.2 ± 3.0	0.69 ± 0.17[a]	327.4 ± 16.3	153.8 ± 31.3
Non-diabetic							
Control	70.4 ± 4.9	75.7 ± 3.3	84.7 ± 10.2	35.0 ± 0.6	1.06 ± 0.12	249.0 ± 11.8	56.3 ± 5.4
62	74.6 ± 12.4	64.5 ± 8.2	60.2 ± 3.8	33.4 ± 0.8	1.90 ± 0.26[a]	223.7 ± 19.3	71.7 ± 6.9

Data are expressed as mean ± S.E.M; n = 6. ANOVA followed by Tukey´s test ($p < 0.05$).
[a]Statistically different from non-diabetic rats.
[b]Statistically different from diabetic control rats.
INS, insulin; Chol, cholesterol; TAG, triacylglycerol; HDL-Chol, high-density lipoprotein-cholesterol; Ins, Insulin; AST, aspartate aminotransferase; and ALT, alanine aminotransferase.

in *C. papaya* leaves could partially explain the pharmacological properties of this plant. Different bioactive phytochemicals found in *C. papaya* possess a wide range of biological activities that can be of valuable therapeutic index. However, the absence or presence of metabolites may be due to differences in polarity of the solvents used for the extraction.

The results of the analysis of *C. papaya* leaves showed that ethanol extract contains a great proportion of steroids, quinones, tannins and alkaloids. There are reports that several drugs have been obtained from alkaloids-containin plants. This feature may be explained by the pharmacological effect of alkaloids, which at low doses have beneficial effects but at higher doses can be toxic (Li et al., 2011). Phytochemical screening of the extracts revealed the presence of different compounds. However, the pharmacological activities of *C. papaya* leaves cannot be determined solely by the result of the phytochemical analysis. Some of these phytocomponents are responsible for the hypoglycemic and hypolipemic effect in diabetic rats. Several studies report that these biological activities might be manifest due to the presence of flavonoids, alkaloids, steroids and quinones (Sanders et al., 2001; Ghosh et al., 2007).

Effect of C. papaya leaf chloroform extract on biochemical parameters

In this study, we observed that the metabolites at greater concentration in the three extracts were steroids. It is known that steroids are molecules that regulate a variety of biological processes, and they possess a high potential, in drugs development, for the treatment of diseases. In addition, their pharmacological properties are diverse; they have analgesic, antipyretic, hypoglycemic, antidepressant, anti-inflammatory, antitumor and anti-rheumatoid properties (Fraile et al., 2012; Trevisan et al., 2012). The insulin deficiency in diabetes *mellitus* is known to stimulate lipolysis in the adipose tissue that gives rise to hyperlipidemia and fatty liver, and it is responsible for a decreased activity of the enzyme lipoprotein lipase, which hydrolyzes lipids, causing a significant increase in serum triacylglyceride concentration. Additionally to its hypoglycemic effect, *C. papaya* chloroform extract also caused a decrease in the concentration of serum triacylglycerides and total cholesterol in diabetic rats. HDL-C levels decreased in diabetes rats without treatment and even more in diabetic rats that received insulin. However, the treatment with different doses of *C. papaya* kept the HDL-C levels similar to those observed in control rats (Table 2). Interestingly, the mechanism by which this decrease in lipid concentration occurs could be explained by stimulation of lipolysis and higher fatty acid utilization. It has also been reported that long-term consumption of chronic α-glucosidase inhibitors improves lipid profiles in animal models of diabetes, suggesting that lower VLDL-triacylglyceride secretion improves hypertriacylglyceridemia and hypercholesterolemia (Standl and Schnell, 2012).

Our study showed that both 31 and 62 mg/kg chloroform extract doses improved AST and ALT levels in diabetic rats, but the 125 mg/kg dose did not change aminotransferase concentrations. The administration of the plant extract to diabetic animals elicited a decrease in aminotransferases in serum (AST and ALT), a finding of clinical and toxicological importance given that changes in their activities are indicative of tissue damage by toxins or symptoms corresponding to hepatic disorders. This reduction of AST and ALT to their normal levels may be favored by the absence of alkanes in the extract. In this regard, it has been found that some parts of *C. papaya* contain large concentrations of alkaloids, which are highly toxic to humans and other animals (Akharaiyi, 2011).

The chloroform extract of *C. papaya* leaf reduced fasting blood glucose levels in STZ-treated rats (Kesari et al., 2005). Accordingly, recent research has been focused on isolation of steroids from plant sources; for instance, those often reported to exhibit anti-diabetic properties and have a relatively low toxicity (Afrose et al., 2009). In agreement with our results

regarding the hypoglycemic effect of *C. papaya* chloroform extract, we consider that steroids hamper the glucose absorption by inhibiting the hydrolyzing enzymes; which could consequently restrict postprandial glucose levels by delaying carbohydrate hydrolysis and intestinal absorption (Hamden et al., 2011; Standl and Shnell, 2012; Liu et al., 2013). It is known that diabetes causes structural and functional changes in intestinal glucose absorption, such as increase of glucose absorption that would cause postprandial hyperglycemia, a major risk factor for the diabetic patient (Hamden et al., 2011). Nevertheless, *C. papaya* chloroform extract had not effect on the fasting plasma insulin level of diabetic rats; this data suggests that *C. papaya* does not stimulate insulin secretion from the remnant β-cells.

A study observed a higher hypoglycemic effect when glibenclamide-pregnenolone was administered to alloxan-treated rats, than glibenclamide alone. This study suggested that a steroid nucleus is important for a high hypoglycemic effect of the glibenclamide-pregnenolone derivative, possibly conditioning the high degree of lipophilicity induced by it (Figueroa-Valverde et al., 2012).

The administration of the chloroform extract of *C. papaya* leaf significantly reversed the damage associated with STZ-induced diabetes, revealing its hypoglycemic and hypolipidemic effects on diabetic rats. From the results obtained, it can be concluded that the hypoglycemic effect of *C. papaya* chloroform extract may be due to its phytoconstituents, especially steroids. The capability of the latter to slow glucose and lipid absorption in the digestive organs represents one of the therapeutic approaches used for the decrease of postprandial hyperglycemia. Taken together, the results suggest that the treatment with the chloroform extract of *C. papaya* could be beneficial in the treatment of hyperglycemia and related hyperlipidemia in diabetes. These results indicate that a delay in carbohydrate absorption improves the effectiveness of subcutaneous insulin. This controls postprandial hyperglycemia in patients with insulin-dependent diabetes *mellitus*, allowing a satisfactory postprandial glycemic control when insulin is administered immediately before ingestion. Thus, an agent such as Acarbose, which delays carbohydrate absorption (Azuma et al., 2006; Liu et al., 2013), may be useful as an adjunct to insulin in the treatment of diabetes *mellitus*.

In conclusion, this study suggests that the high concentrations of steroids in *C. papaya* leaves could be responsible for the hypoglycemic and hypolipidemic effect of the chloroform extract in diabetic rats. Nevertheless, more studies are required to identify, isolate, and study these molecules to characterize the observed pharmacologic activities of this plant. Our findings indicate that *C. papaya* leaf extract may be beneficial to diabetic patients and helpful in the prevention of diabetic complications by dyslipidemia improvement.

Authors' contributions

JCDZ, IEJR and DYBO contributed the design and conducted the study, collection, analysis, interpreting of data and writing of the manuscript. DEAG (MS student) contributed in collecting plant sample and identification, and carrying out biochemistry analysis. LFRF and CELG performed qualitative and quantitative determination. LLM contributed in acquisition of funding and critically revising the manuscript. CATZ and JLBC performed statistical analyses and manuscript preparation. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgment

This study was financially supported by the Program for Research, Support and Consolidation of Academic Groups (PFICA, acronym in Spanish), UJAT-2010-C06-10 grant.

REFERENCES

Afrose, S., Hossain, M.S., Maki, T., Tsujii, H., 2009. Karaya root saponin exerts a hypocholesterolemic response in rats fed a high-cholesterol diet. Nutr. Res. 29, 350-354.

Ahmad, N., Fazal, H., Ayaz, M., Abbasi, B.H., Mohammad, I., Fazal, L., 2011. Dengue fever treatment with *Carica papaya* leaves extracts. Asian Pac. J. Trop. Biomed. 1, 330-333.

Akharaiyi, F.C., 2011. Antibacterial, phytochemical and antioxidant activities of *Datura metel*. Int. J. Pharm. Tech. Res. 3, 478-483.

Aruoma, O.I., Hayashi, Y., Marotta, F., Mantello, P., Rachmilewitz, E., Montagnier, L., 2010. Applications and bioefficacy of the functional food supplement fermented papaya preparation. Toxicology 278, 6-16.

Azarkan, M., Garcia-Pino, A., Dibiani, R., Wyns, L., Loris, R., Baeyens-Volant, D., 2006. Crystallization and preliminary X-ray analysis of a protease inhibitor from the latex of *Carica papaya*. Acta Crystallogr. Sect. F. Struct. Biol. Cryst. Commun. 62, 1239-1242.

Azuma, K., Toyofuku, Y., Iesaki, T., Otsuka, A., Tanaka, A., Mita, T., Hirose, T., Tanaka, Y., Daida, H., Kawamori, R., Watada, H., 2006. Acarbose, an alpha-glucosidase inhibitor, improves endothelial dysfunction in Goto-Kakizaki rats exhibiting repetitive blood glucose fluctuation. Biochem. Biophys. Res. Commun. 345, 688-693.

Barreto, M.C., 2005. Lipid extraction and cholesterol quantification - a simple protocol. J. J. Chem. Educ. 82, 103.

Canini, A., Alesiani, D., D'Arcangelo, G., Tagliatesta, P., 2007. Gas chromatography-mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. J. Food Compos. Anal. 20, 584-590.

Courteix, C., Bardin, M., Chantelauze, C., Lavarenne, J., Eschalier, A., 1994. Study of the sensitivity of the diabetes-induced pain model in rats to a range of analgesics. Pain 57, 153-160.

Danese, C., Esposito, D., D'Alfonso, V., Cirene, M., Ambrosino, M., Colotto, M., 2006. Plasma glucose level decreases as collateral effect of fermented papaya preparation use. Clin. Ter. 157, 195-198.

De D, Ali K.M., Chatterjee. K., Bera, T.K., Ghosh, D., 2012. Antihyperglycemic and antihyperlipidemic effects of *n*-hexane fraction from the hydro-methanolic extract of sepals of *Salmalia malabarica* in streptozotocin-induced diabetic rats. J. Complement. Integr. Med. 9, 1553-3840.

Fazel, S., Hamidreza, M., Rouhollah, G., Mohammadreza, V., 2008. Sprectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J. Pharm. Sci. 32, 17-20.

FHEUM, 2001 Farmacopea Herbolaria de los Estados Unidos Mexicanos,. Secretaria de Salud. 10th ed. México.

Figueroa-Valverde, L., Díaz-Cedillo, F., López-Ramos, M., García-Cervera E, Pool-Gómez E, Cardena-Arredondo C, Ancona-León G. 2012. Glibenclamide-pregnenolone derivative has greater hypoglycemic effects and biodistribution than glibenclamide-OH in alloxan-rats. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc. Czech. Repub. 156, 122-127.

Fraile, L., Crisci, E., Córdoba, L., Navarro, M.A., Osada, J., Montoya, M., 2012. Immunomodulatory properties of beta-sitosterol in pig immune responses. Int. Immunopharmacol. 13, 316-321.

Ghosh, D., Konishi, T., 2007. Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. Asia Pac. J. Clin. Nutr. 16, 200-208.

Gupta, S., Kataria, M., Gupta, P.K., Murganandan, S., Yashroy, R.C., 2004. Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. J. Ethnopharmacol. 90, 185-189.

Hamden, K., Jaouadi, B., Zaraî, N., Rebai, T., Carreau, S., Elfeki, A., 2011. Inhibitory effects of estrogens on digestive enzymes, insulin deficiency, and pancreas toxicity in diabetic rats. J. Physiol. Biochem. 67, 121-128.

Head, W.F. Jr, Lauter, W.M., 1957. Ultrasonic depolymerization of natural polymers. J. Am. Pharm. Assoc. Am. Pharm. Assoc. 46, 617-621.

ISO, 1988. Sorghum: Determination of Tannin Content. ISO 9648, International Organization for Standardization.

Juárez-Rojop, I., Díaz-Zagoya, J.C., Ble-Castillo, J.L., Miranda-Osorio, P., Castell-Rodríguez, A.E., Tovilla-Zárate, C.A., Rodríguez-Hernández, A., Aguilar-Mariscal, H., Ramón-Frías, T., Bermúdez-Ocaña, D.Y., 2012. Hypoglycemic effect of *Carica papaya* leaves in streptozotocin-induced diabetic rats. BMC Comp. Alt. Med. 12, 236

Kesari, A.N., Gupta, R.K., Watal, G., 2005. Hypoglycemic effects of *Murraya koenigii* on normal and alloxan-diabetic rabbits. J. Ethnopharmacol. 97, 247-251.

Khuzhaev, V.U., Aripova, S.F., 2000. Pseudocarpaine from *Carica papaya*. Chem. Nat. Comp. 36, 418-420.

Koehn, F.E., Carter, G.T., 2005. The evolving role of natural products in drug discovery. Nat. Rev. Drug Discov. 4, 206-220.

Li, N., Xia, Q., Ruan, J., Fu, P.P., Lin, G., 2011. Hepatotoxicity and tumorigenicity induced by metabolic activation of pyrrolizidine alkaloids in herbs. Curr. Drug Metab. 12, 823-834.

Lim, T.K., 2012. *Carica papaya*. *In: Edible Medicinal and Non-Medicinal Plants*. Springer; 1:693-717.

Liu, S.Z., Deng, Y.X., Chen, B., Zhang, X.J., Shi, Q.Z., Qiu, X.M., 2013. Antihyperglycemic effect of the traditional Chinese scutellaria-coptis herb couple and its main components in streptozotocin-induced diabetic rats. J. Ethnopharmacol. 145, 490-498.

Miean, K.H., Mohamed, S., 2001. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. J. Agric. Food Chem. 49, 3106-3112.

Owoyele, B.V., Adebukola, O.M., Funmilayo, A.A., Soladoye, A.O., 2008. Anti-inflammatory activities of ethanolic extract of *Carica papaya* leaves. Inflammopharmacol. 16, 168-173.

Pochapski, M.T., Fosquiera, E.C., Esmerino, L.A., Dos Santos, E.B., Farago, P.V., Santos, F.A., Groppo, F.C., 2011. Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves' extract from *Ipomoea batatas* (L.) Lam. Pharmacogn. Mag. 7, 165-170.

Polonsky, K.S., 2012. The past 200 years in diabetes. N. Engl. J. Med. 367, 1332-1340.

Robin, E.D., 1945. The reaction of vitamin A with Lieberman-Burchard reagent. Science 102, 17.

Sanders, R.A., Rauscher, F.M., Watkins, J.B., 2001. Effects of quercetin on antioxidant defense in streptozotocin-induced diabetic rats. J. Biochem. Mol. Toxicol. 15, 143-149.

Sasidharan, S., Sumathi, V., Jegathambigai, N.R., Latha, L.Y., 2011. Antihyperglycaemic effects of ethanol extracts of *Carica papaya* and *Pandanus amaryfollius* leaf in streptozotocin-induced diabetic mice. Nat. Prod. Res. 20, 1982-1987.

Standl, E., Schnell, O., 2012. Alpha-glucosidase inhibitors: cardiovascular considerations and trial evaluation. Diab. Vasc. Dis. Res. 9, 163-169.

Trevisan, G., Rossato, M.F., Walker, C.I., Klafke, J.Z., Rosa, F., Oliveira, S.M., Tonello, R., Guerra, G.P., Boligon, A.A., Zanon, R.B., Athayde, M.L., Ferreira, J., 2012. Identification of the plant steroid α-spinasterol as a novel transient receptor potential vanilloid 1 antagonist with antinociceptive properties. J. Pharmacol. Exp. Ther. 343, 258-269.

WHO, 2012. *Traditional medicine strategy*: 2002-2005. World Health Organization press, p. 1-6.