

Kalanchoe daigremontiana: Functional Properties and Histopathological Effects on Wistar Rats under Hyperglycemia-inducing Diet

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ABSTRACT

Introduction: Diabetes is a worldwide pandemic disease with an expensive way of control. Locals around the world have been used botanical extracts to reduce the effects of this deadly disease to improve the quality of life of the patients. **Objectives:** To evaluate the effects of crude extracts of the ethnobotanical species *K. daigremontiana* on a hyperglycemia rat model to support its antidiabetic use in traditional medicine. **Materials and Methods:** Crude leaves extract were daily administered *per os* to Wistar rats for six months. Hyperglycemic induction model was rich-sucrose diets. Six animals per group were used: i) standard diet, ii) sucrose-rich diet, iii) sucrose-rich diet + extract (1 ml), iv) sucrose-rich diet + extract (2 ml) and v) standard diet + extract (2 ml). All animals were sacrificed and the liver prepared for histopathological analysis. Powdered leaves were functionally characterized and analyzed by Fourier Transform Infrared (FTIR) spectroscopy. **Results:** Treatment with 2 ml of the crude extract caused the least tissue damage, compared to the control. A high antioxidant activity (DPPH: 111.14 μ M Trolox equivalents g-1 dry weight (DW); ABTS: 223.90 μ M Trolox equivalents g-1 DW) was observed. A content of 0.54 mg ascorbic acid g-1 DW, total phenol content of 17.45 mg gallic acid g-1 DW and flavonoid content of 2.3 mg equivalent quercetin g-1 DW were determined. No conclusive evidence on the molecules present in the extract was obtained by FTIR spectroscopy. **Conclusion:** The use of dosages of 2 ml of crude extract of *Kalanchoe daigremontiana* reduces liver damage under high sucrose conditions.

Key words: Traditional medicine, Anti-hyperglycemic, Diabetes Sucrose-rich diet, *Kalanchoe daigremontiana*, Phytotherapy.

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INTRODUCTION

Diabetes has become one of the greatest epidemics of the 21st century. The World Health Organization (WHO) estimated that diabetes incidence has tripled since 1995 and today more than 347 million people worldwide live with diabetes.¹ According to the International Diabetes Federation (IDF),² the countries with

the highest diabetes incidence are China, India, the United States, Brazil, Russia and Mexico (in that order). Diabetes is a chronic disease with complex etiology and severe consequences both for the patient and society. Therefore, a comprehensive, affordable and human treatment is urgently needed. One possible approach is the use of



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extracts of *Kalanchoe daigremontiana* Raym.-Hamet and H. Perrier (*Crassulaceae*).³ The use of this plant in traditional medicine is well known, especially in North and South America.⁴

According to the International Diabetes Federation (IDF), the countries with highest diabetes incidence are China, India, the United States, Brazil, Russia and Mexico (in that order). Mexico ranks eighth in diabetes prevalence. The country will occupy the sixth or seventh place by 2025, with 11.9 million Mexicans with diabetes. Since 2000, diabetes is the leading cause of death in Mexico, accounting for 17.2% of total mortality.¹ The National Nutrition Survey 2012 showed that 9.17% of the adult population was diagnosed with diabetes, i.e. 6.4 million people.

In México, 15.78% of diabetes patients lack social security and almost two-thirds of them are treated by private medics. Among patients covered by social security, nearly 24% receive treatment in the Social Protection System of Health and the rest attend the Mexican Social Security Institute (IMSS). The direct cost of diabetes for 20-79 years-old patients ranges from 232 to 422 billion dollars per year worldwide. If diabetes incidence grows as predicted, by 2025 this cost will be 303-559 billion dollars. In Mexico, the total cost of diabetes grew from 2.97 billion Mexican pesos in 2003 to 8.84 billion in 2010.⁵

Being diabetes a chronic disease with complex etiology and severe consequences both for the patient and society, a comprehensive, affordable and human treatment is urgently needed. One possible approach is the use of extracts of *Kalanchoe daigremontiana* Raym.-Hamet and H. Perrier (*Crassulaceae*).⁶⁻⁸

The use of this plant in traditional medicine is well known, especially in North and South America.⁹⁻¹³ While the use of medicinal plants was widespread two centuries ago, it fell into disuse with the advent of modern pharmacy. Nevertheless, the interest in herbal medicines as a therapeutic alternative has revived due in part to the inaccessibility to health services among poorer population, along with a lack of access to drugs and the high costs involved. Currently, many plant species are under study to identify and isolate substances for therapeutic use and to produce nutraceutical or functional products, to prevent diseases.

The use of medicinal plants is deeply rooted in Mexico. Specifically, ethno-botanical research has recorded about 300 species with hypoglycemic properties. This traditional use, however, is limited by the lack of knowledge on the active substance and its effects and on the therapeutic dose and by the likely occurrence of side effects and toxicity. The wide variations in

preparation methods, administration routes and the physical properties of the drugs further complicate the safe and efficacious use of medicinal plants.

Kalanchoe daigremontiana is a perennial, glabrous herb,¹⁴ commonly known as “Mother-of-Thousands”; it reproduces by developing whole plantlets on leaves.¹⁵ Originally native to South Africa, India, China and Brazil, numerous *Kalanchoe* species are widely distributed in tropical and subtropical areas.¹⁶ Native to Madagascar, *K. daigremontiana* is considered a noxious invasive plant in arid zones. It is commonly found in disturbed sites of the semiarid northeastern region of Guanajuato and also in Querétaro and Hidalgo, Mexico (Figure 1).

Different types of secondary metabolites have been isolated from *Kalanchoe* spp. in recent decades. Among them are alkaloids,^{17,18} anthraquinones and bufadienolides;¹⁹ carbazoles, coumarins and lactones;^{20,21} lignans, phenyl naphthalene, quassinoids and saponins;^{22,23} stivenes and terpenes;²⁴⁻²⁷ Antitumor,²⁴ antihistaminic, anti-inflammatory,²⁸ and immunomodulatory^{29,30} effects have been reported for some species of the genus. Flavonoid glycosides are vegetable pigments with antioxidant capacity and bufadienolides are cardioactive steroids whose efficacy against heart disease was already known to the ancient Egyptians.³¹

An aqueous preparation from *K. pinnata* L. (*Crassulaceae*) was reported to show a hypoglycemic effect,³² and a similar effect was found in an ethanol extract of the plant, observing decreased blood sugar levels and improvements in morphology and number of B cells in Langerhans islets.³³

Various works have studied the number, properties and type of molecules involved in the therapeutic effects of *Kalanchoe* spp. This study is aimed to provide scientific support to the traditional use of *K. daigremontiana* as an antidiabetic, characterizing the functional properties



Figure 1: *Kalanchoe daigremontiana* specie.

Shrub type plant, from 20 cm to 50 cm high. Petiolate, triangular, oblong-lanceolate leaves with serrated margin. From 1 to 3 cm wide by 4 to 5 long. Simple stems, erect, rounded, 1 to 2 cm thick.

of powdered leaves, determining its composition by functional group analysis and assessing *in vivo* the biological activity (histopathological effects) of a crude *K. daigremontiana* extract on a hyperglycemia model.

MATERIALS AND METHODS

Plant material collection and species identification

K. daigremontiana specimens were collected (during March 2015) by Dr. Alma Delia Hernández-Fuentes in Ulapa de Melchor Ocampo, Tetepango, Hidalgo, in Central Mexico, at -99.167778 longitude, 20.142500 latitude (GPS coordinates). Traditionally, local people have used this plant species in their traditional healthcare system. Whole plants (roots, leaves and stems) were collected and prepared for the transportation. Species identification was performed in the laboratory of botany at the Institute of Biological Sciences of the Universidad Autónoma del Estado de Hidalgo. A voucher specimen representing the plant material was deposited in the herbarium of the Institute of Biological Sciences of the Universidad Autónoma del Estado de Hidalgo and the identification code 06 was assigned.

Characterization of the functional properties of the leaves

To characterize the functional properties of *K. daigremontiana* leaves, the following parameters were assessed: ascorbic acid content, total phenolic content, flavonoid content and antioxidant activity.

The ascorbic acid content was determined according to Klein and Perry,³⁴ total phenol and flavonoid contents were determined after extraction with methanol, as described by Rosales *et al.*³⁵ with slight modifications. Antioxidant activity was determined according to Brand-Williams *et al.*³⁶ as the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. The antioxidant capacity was determined by the Trolox equivalent method (TEAC) as described by Re *et al.*³⁷

Functional group analysis

Fourier Transform Infrared (FTIR) spectroscopic analysis

For the FTIR studies, dried *K. daigremontiana* (50 g) of leaves were cut into small pieces and soaked in 250 ml of reagent-grade methanol for seven days. The methanol solution was filtered and concentrated under vacuum using a rotary evaporator (BÜCHI R-210, Flawil, Germany). The functional groups in the methanol extract of *K. daigremontiana* were determined by FTIR spectroscopy (Perkin-Elmer GXFT-IR, Waltham, MA). An absorption spectrum was recorded in the range

4000-400 cm^{-1} at room temperature. The sample was prepared using the standard KBr pellet method.

Biological activity

Preparation of the extract

Harvested plants were rinsed three times with sterile distilled water and the leaves were collected. Leaves were dusted off, cleaned, allowed to dry in the shade and weighed. Leaves were macerated without any solvent (dry milled) in a commercial juice extractor (UNIWORL UJC-N50 model, Bell, CA) to obtain the extract. The crude extract was filtered through a Whatman grade 40-filtration paper. An average of 230 ml of filtered crude extract was obtained per every 500 g of plant leaves.

Experimental animals

Male Wistar albino rats weighing 190-200 g bred in the Vivarium of the Institute of Health Sciences, Universidad Autónoma del Estado de Hidalgo (Pachuca, Hidalgo, Mexico) were used in this study. The Bioethics Committee of the Institute of Health Sciences approved the research protocol and the identification code 202014 was assigned.

All animals were housed in polypropylene cages ($43 \times 27 \times 15$ cm); each cage lodged no more than six animals. The animals were kept in an isolation room at 17°C , 30% RH, under a 12 h light/dark cycle. All animals were acclimatized for two weeks before experimental use. All guidelines established in the Mexican Official Norm (NOM-062-ZOO-1999)³⁸ concerning the production, care and use of laboratory animals were followed, as well as the procedures for the Care and Use of Laboratory Animals.³⁹

Induction of hyperglycemia

To induce experimental hyperglycemia, rats were fed with a sucrose-rich diet (SuRD). To this end, a standard pellet diet (Global Teklab™ food for rodents, 18% protein, sterilizable) was supplemented with a 30% sucrose solution (300 g of pure sucrose in 1 L of sterile water); both components were allowed *ad libitum*.

Experimental setup

The animals were distributed into five groups as described below. *K. daigremontiana* extract was administered daily through an intra-gastric gavage to prevent degradation of active components. The animals were allowed water *ad libitum* and were fed with their respective diet for six months. Individual weight gain was evaluated during this period by recording individual rat weight once a week. Rats were distributed as follows: Group I: Normal control. Animals were fed with standard pellet diet and

sterilized water *ad libitum*. Group II: Negative control. Untreated, hyperglycemia-induced rats. Group III. Positive control. Animals were fed with standard diet + 2 ml of *K. daigremontiana* extract (preventive effect). Group IV: Animals fed with SuRD + 1 ml of *K. daigremontiana* extract (therapeutic effect). Group V: Animals fed with SuRD + 2 ml of *K. daigremontiana* extract (therapeutic effect).

After six months, when significant differences in weight gain among groups were evident, the animals were humanely sacrificed and the liver was retrieved for macroscopic examination and histological studies.

Histopathology

Liver samples from each group were fixed in 3.8% formaldehyde phosphate buffered aqueous solution for at least 24 hr. Then, samples were included in paraffin inclusion and an automated tissue processor (Micom, model TP1020, Thermo Fisher Scientific™) was used. Four-micrometer-thick slices were obtained in a manual microtome (Leica™, model 2125RT, Nussloch, Germany) and stained with hematoxylin-eosin (HE).⁴⁰ Histological preparations were analyzed with a light-field microscope (Olympus™, model BX-41, Waltham, MA). Images were taken with a digital camera (Evolution VF™, Media Cybernetics, Rockville, MD) and processed with the software Image Pro Express v.6.0 (Media Cybernetics).

Statistical analysis

The results were analyzed by one-way ANOVA and a Tukey-Kramer *post hoc* test. Differences between groups were regarded as significant when $P < 0.05$.

RESULTS

Functional characterization of leaves

K. daigremontiana powdered leaves showed a content of 0.54 mg of ascorbic acid/ DW, a total phenol content of 17.45 mg gallic acid/g DW and a flavonoid content of 2.3 mg of equivalent quercetin/g DW. Concerning antioxidant activity, 111.14 μM Trolox/g DW and 223.90 μM Trolox/g were determined by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and ABTS ((2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), respectively. Results are summarized in Table 1.

Functional group analysis

FTIR spectroscopy was used to identify the functional groups in a *K. daigremontiana* methanol extract. A representative spectrum is shown in Figure 2. The presence of -OH vibration strain bands from different

chemical environments was evident in the 3600-3100 cm^{-1} interval. A prominent band observed at 3424 cm^{-1} corresponded to hydroxyl groups. Bands in the 2973-2917 cm^{-1} interval are associated with C-H stretching vibrations from CH_2 and CH_3 . The band at 1625 cm^{-1} can be attributed to amines. The intense band at 1051 cm^{-1} was assigned to C-O bond stretching vibration.⁴¹ The band from 831 to 770 cm^{-1} represents the C-H banding region, out of the plane, with signals of low intensities. Results are summarized in Table 2.

Biological activity

Hyperglycemia induction

Table 3 summarizes the total weight gain in rats after six months of a sucrose-rich diet. Statistically significant differences in weight gain were observed between experimental groups. Positive control (animals fed with SuRD) averaged 599 g, while negative control (animals under standard diet) averaged 548 g. These results suggest that the method to induce hyperglycemia and its relation with overweight are useful for this study.

Animals fed with standard diet and treated with 2 ml of crude *K. daigremontiana* showed the lowest mean weight. This could indicate the effectiveness of the crude extract for preventive purposes. No significant differences in final mean weight were observed between animals fed with SuRD and treated with 1 or 2 ml of the crude extract.

Histopathology

The results are summarized in Table 4 and Figure 3. Animals in the negative control group, fed with SuRD with no extract supplement (Group I) showed hepatocytes with a significant number of vacuoles and many hepatocytes exhibited hollow spaces (Figure 3A) [moderate damage, Table 3].

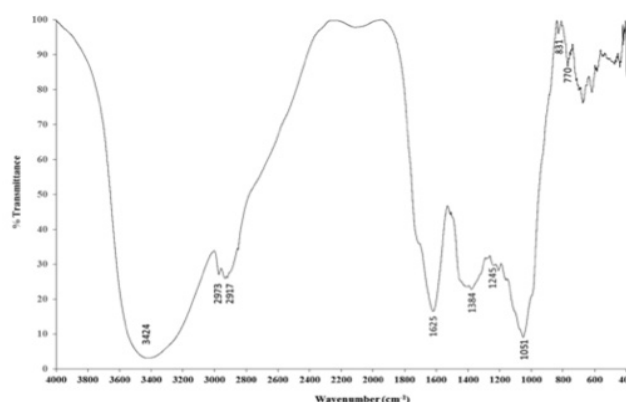


Figure 2: Fourier transform infrared spectrum of methanolic leaf extract of *Kalanchoe daigremontiana*.

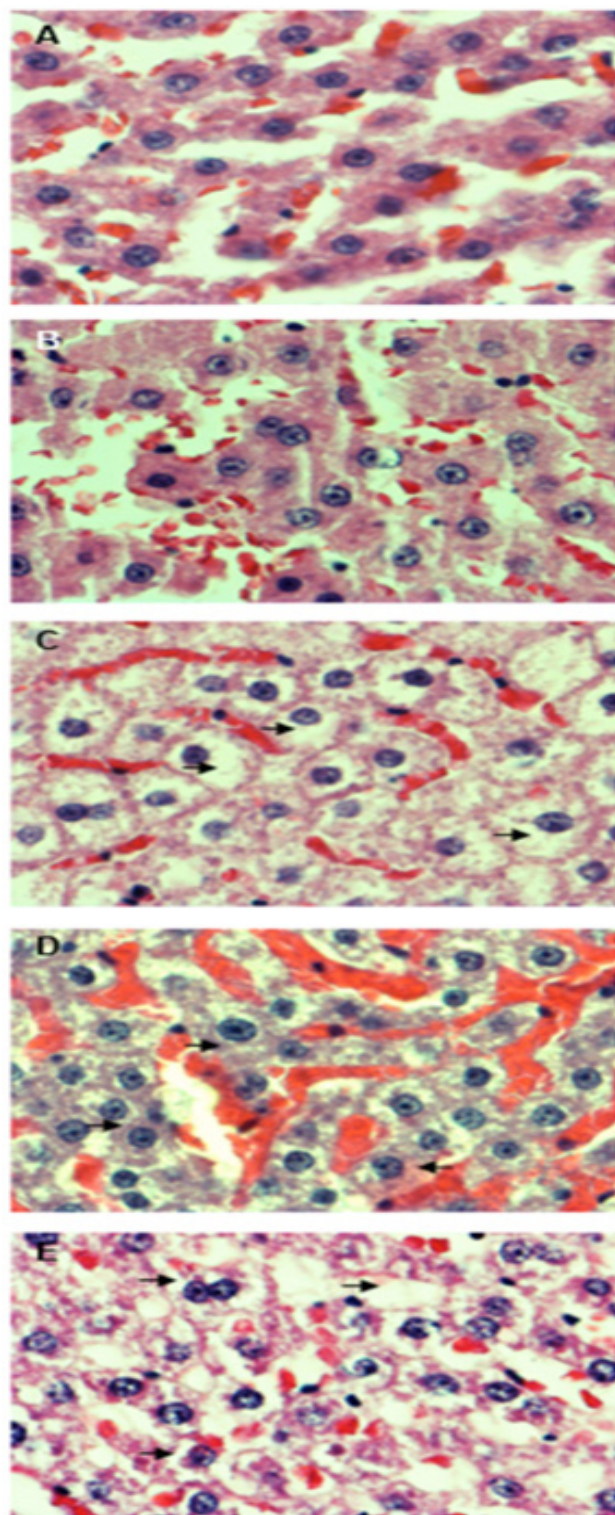


Figure 3: Histopathological changes in Wistar rats liver under a high sucrose diet treated with *Kalanchoe daigremontiana* crude extracts.

3A. Negative control (sugar-rich diet). The hepatocytes are observed with large empty spaces in their cytoplasm and several of them have lost their characteristic polyhedral shape (arrows). 3E. Positive control (standard diet). Changes in hepatocytes are minimal. 3B. SD + 2 mL (standard diet + 2 mL of extract). Changes in hepatocytes are minimal. 3C. SuRD + 1 mL (sugar-rich diet + 1 mL of extract). The presence of numerous vacuoles (arrows) in the cytoplasm of hepatocytes is observed. 2D. SuRD + 2 mL (sugar-rich diet + 2 mL of extract). Normal tissue architecture. There are large numbers of vacuoles in hepatocytes.

Table 1: Content of carotenoids, ascorbic acid, total phenols, total flavonoids and antioxidant activity by by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and ABTS ((2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) in *Kalanchoe daigremontiana* leaves.

Function	Values
Red carotenoids (mg g ⁻¹ DW)	0.39 ± 0.011
Yellow carotenoids (mg g ⁻¹ DW)	0.46 ± 0.010
Total carotenoids (mg g ⁻¹ DW)	0.85 ± 0.014
Ascorbic acid (mg g ⁻¹ DW)	0.54 ± 0.21
Total phenols (mg GAE g ⁻¹ DW)	17.35 ± 0.12
Flavonoids total (mg QE g ⁻¹ DW)	21.40 ± 1.50
DPPH (µM TE g ⁻¹ DW)	110.85 ± 0.36
ABTS (µM TE g ⁻¹ DW)	222.43 ± 3.43

Table 2: Major functional groups observed in the Fourier transform infrared spectra of methanolic leave extract.

IR spectral values (cm ⁻¹)	Bond and functional groups	Probable phytochemicals
3424	O-H	Polyphenols, tannins
2973	(CH ₃ , CH ₂ , CH) alkanes	Flavonoids and polyphenols
1625	(NH ₂ scissoring) amines	Alkaloids, flavonoids and polyphenols
1384	(CH ₃ , CH ₂) alkanes, O-H bending	Flavonoids, tannins and polyphenols
1245	O-C carboxylic acid derivates	Flavonoids, tannins and carboxylic acid derivates
1051	O-H alcohols and phenols	Flavonoids, polyphenols
831-770	=C-H	Flavonoids, tannins and polyphenols

Animals fed with standard diet (positive control) showed normal liver tissue architecture, with hepatocytes exhibiting clear vacuoles and some albuminous degeneration (due to sample collection time) (Figure 3E).

No differences were observed in animals fed with standard diet supplemented with 2 ml of the crude extract (preventive effect, Group III, Figure 3B) with respect to normal controls.

Animals fed with SuRD supplemented with 1 ml of crude extract (curative effect, group IV) showed normal liver tissue architecture. However, the cytoplasm of some hepatocytes exhibited abundant vacuoles, while other cells had a normal appearance, in variable proportions (Figure 3 C).

Animals in group V (curative effect), fed with SuRD and treated with 2 ml of crude extract showed a normal liver

Table 3: Total weight gain after six months of sucrose-rich diet in rats treated with crude *Kalanchoe daigremontiana* extract.

Treatment	Average weight (g)
Negative control (sugar-rich diet)	599 ^a
Normal control (standard diet)	548 ^b
SD + 2 ml (standard diet + 2 ml of extract)	418 ^c
SuRD + 1 ml (sugar-rich diet + 1 ml of extract)	565 ^d
SuRD + 2 ml (sugar-rich diet + 2 ml of extract)	596 ^d

Letters indicate significant differences (with $\alpha = 0.05$, Tukey-Kramer test)

Table 4: Histological changes in liver tissues.

Groups	Liver damage	Vacuolation	hepatocytes
I. Negative control (sugar-rich diet)	@@	+++	###
II. Normal control (standard diet)	0	+	#
III. SD + 2 ml (standard diet + 2 ml of extract)	0	+	#
IV. SuRD + 1 ml (sugar-rich diet + 1 ml of extract)	0	+++	##
V. SuRD + 2 ml (sugar-rich diet + 2 ml of extract)	0	+++	##

Liver tissue damage: 0 = No damage. @ = Mild damage. @@ = Moderate damage. @@@ = Marked damage. Vacuolation: + = normal presence of vacuoles. ++ = Moderate high presence of vacuoles. +++ = Significant high presence of vacuoles. Hepatocytes: # Normal appearance. ## = Moderate damage. ### = Marked damage.

tissue architecture, but several hepatocytes exhibited vacuoles, while other cells had a normal appearance (Figure 3D).

DISCUSSION

Diabetes is becoming a global pandemic and coping with it requires inexpensive and effective means to improve the patients' quality of life. This goal could be achieved by using plant extracts. Several protocols and techniques have been developed to determine the usefulness and mechanisms of action of herbal remedies. The phytochemical characterization of the extract is a mandatory step, followed by testing the effectiveness of the extract *in vivo*. Thus, a reliable biological model of the disease, diabetes in this case, is required. One of the most commonly reported methods for diabetes induction in rats involves the use of streptozotocin

(STZ). The effects of STZ are variable and usually are observed after a period of 15 days-8 weeks.⁴²⁻⁴⁶

In contrast, the effects of SuRD administration seem to be closer to the pathogenesis of the disease and that is why it was used in this study. SuRD administration is a reliable method to produce metabolic disorders.⁴⁷ The results herein reported are similar to those found by Long *et al.*⁴⁸ that SuRD induced mild glucose intolerance after two months of treatment followed by "temporally no symptom phase (after three months) and then produced significant metabolic abnormality." Accordingly, we expected the *in vivo* studies to last about three months. Rat body weight varied significantly during this period, but it was until month 6 that overweight was observed. The slow progression of this metabolic disorder is also representative of the natural process of the disease in humans, so it is likely that the effect of *K. daigremontiana* extract is also more representative.

The content of carotenoids in the leaves of *Kalanchoe daigremontiana* was lower than that reported by Santiago-Saenz *et al.*⁴⁹ in purslane (*Portulaca oleracea* L.), quelite ash (*Chenopodium berlandieri* L.) and quintonil (*Amaranthus hybridus* L.). In this regard, it is mentioned that carotenes protect the cells and tissues of highly oxidizing radicals and singlet oxygen, through their antioxidant activity Cosme *et al.*⁵⁰ However, the content of ascorbic acid was higher than that reported for quality ash and quintonil. This vitamin is a powerful antioxidant useful for the elimination of free radicals from the human body. In nutritional terms, the recommended minimum requirement of vitamin C for an adult is 60 mg.⁵¹ In relation to the content of total phenols and flavonoids, this content was higher in *K. daigremontiana* than reports by Santiago-Saenz *et al.*⁴⁹ This is important because total phenols and flavonoids are useful to treat diseases related to inflammatory processes and cardiovascular disorders due to the activity they exert on the human circulatory system.⁵² The ABTS test showed a higher value than that obtained by DPPH, which indicates that the ABTS test is better for matrices such as fruits, vegetables and oils.

Obesity is a complex metabolic disorder, associated to diseases such as cirrhosis, non-alcoholic fatty liver disease and diabetes Type 2. In humans and rodents, obesity promotes hepatic steatosis and inflammation.⁵³ Since obesity is characterized by an excessive body fat accumulation, diabetes type 2 and obesity are both associated with hypertension and hyperlipidemia.⁵⁴ In our experiment, after six months of SuRD administration, the negative control group showed the highest mean weight, indicating overweight and possibly a propensity to diabetes.

Our results agree with those reported by Kurup and Mini.⁵⁵ Histopathological study showed the effectiveness of an extract from *Averrhoa bilimbi* L. (*Oxalidaceae*) to efficiently prevent oxidative damage in hepatocytes without affecting the cellular structural integrity in STZ-induced diabetic rats, possibly by activating tissue antioxidants. Soliman *et al.*⁵⁶ reported that extracts from *Origanum majorana* L. (*Lamiaceae*) reverted histopathological changes in liver and kidney by decreasing lipid accumulation and increasing the regeneration of hepatic parenchyma, restoring normal renal architecture and removing fat droplets. Hazarika *et al.*⁵⁷ observed lipid droplets in the liver and an increase in adipocyte size in Wistar albino rats fed with a high-carbohydrate diet, concluding that such diet caused ultrastructural abnormalities. In our experiment, normal controls presented normal liver tissue architecture, while negative controls showed numerous vacuolated hepatocytes. This is in agreement with the study by Kurup and Mini⁵⁵ cited above.

Histopathological damage in pancreas, liver and kidney secondary to diabetes improved after treatment with hydroalcoholic leaf extract from *Citrullus colocynthis* L. (*Cucurbitaceae*) in STZ-induced diabetic rats. It was also concluded that *C. colocynthis* leaf extracts might have a protective effect on liver, kidney and pancreas.

Sirovina *et al.*⁵⁸ reported that administering naringenin (a flavonoid found in grapefruit, orange and tomato fruits) resulted in a significant decrease in lipid peroxidation in liver and kidney tissue, as well as in a reduced number of vacuolated liver cells and in the degree of vacuolization. Abdelaziz and Ali⁵⁹ reported the protective effect of an aqueous suspension of *Phoenix dactylifera* L. (*Araceae*) seeds against chemically induced hepatic injury in rats. Liver histopathologic examination showed that *P. dactylifera* seeds decrease the incidence of liver lesions (including vacuolization and fibroblast proliferation); additionally, Gupta *et al.*⁶⁰ demonstrated by liver histopathological examination that pioglitazone induced pathological liver damage. Further studies on liver tissues support its significant hepatoprotective activity on acetaminophen-induced hepatotoxicity.

Our results are in agreement with those reported by Sipal *et al.*⁶¹ where diabetic rats treated with olmesertan exhibited regular hepatocyte plates. According to Bansal *et al.*⁶² a flavonoid from *Pilea microphylla* (L.) Liebm. (*Urticaceae*) preserved islet architecture and prevented hepatocyte hypertrophy.

The biological activity of *K. daigremontiana* extract can be explained by its functional characterization. Yuliani *et al.*³³ reported oxidative stress to play a key role in the pathogenesis of diabetes mellitus type

2. Supplementation with curcuminoids (natural polyphenols) was reported to exert an antioxidant effect in diabetic individuals and points to the need to investigate the impact of these antioxidant effects on diabetes and its complications. According to Yuliani, hyperglycemia is characterized by an increase in oxidative stress, which leads to an insufficiency in insulin secretion. We suggest that the reduction in liver cellular damage by *K. daigremontiana* extract is related to the antioxidant effect of constituents like quercetin (herein found in *K. daigremontiana* leaves). This agrees with the results reported by Iskender *et al.*⁶³ that hesperidin and quercetin increased oxidative stress, while SIRT1 (NAD-dependent sirtuin-1 deacetylase) levels decreased in a STZ-induced diabetes experimental model. Almogbel and Rasheed⁶⁴ suggested an association between protein oxidation and diabetes progression. They concluded that protein-mediated oxidative stress could be useful when evaluating diabetes progression in human patients. On the other hand, Ganjifrockwala *et al.*⁶⁵ concluded that early management with an antioxidant-rich diet and lifestyle changes would help diabetes mellitus type 2 patients to overcome the debilitating complications of the disease.

The proposed model to assess *in vivo* the capacity of *K. daigremontiana* crude extract to reduce the effects of a sucrose-rich diet (diabetes promoter) could not be compared with other models, since no comparable experiments were found in the literature.

CONCLUSION

Considering that the administration of 2 ml of *K. daigremontiana* crude extract to SuRD-fed rats decreased hepatic damage with respect to untreated controls and considering that functional analysis showed a high antioxidant activity in the extract which could be related to its biological activity, our findings provide a scientific rationale for the use of *K. daigremontiana* leaf extracts as a promising agent to prevent liver injury in diabetes. This contributes to validate the traditional use of *K. daigremontiana* as an antidiabetic. To the best of our knowledge, there are no reports in the literature on scientific confirmation of the traditional use of *Kalanchoe daigremontiana* in the recovery of hepatic damage caused by high sugar consumption. These are promising albeit preliminary results, meriting further studies.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ABBREVIATIONS

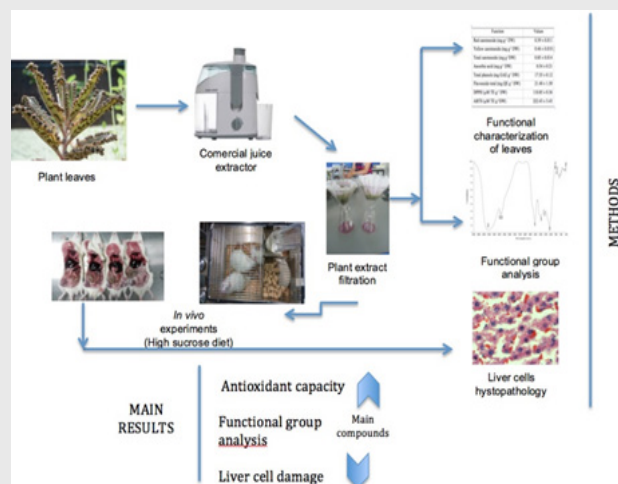
SD: Standar diet; **SuRD:** Sugar rich diet.

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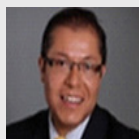
PICTORIAL ABSTRACT



SUMMARY

Process for the *Kalanchoe daigremontiana* test against hyperglycemia. Extraction of sap from mature plants. Obtention of functional characteristics. Induction of hyperglycemia in laboratory animals. Feeding rats with pure extract in hyperglycemic rats. Histopathological comparison of healthy / damaged livers. Improvement of damaged livers supplied with *Kalanchoe daigremontiana* sap compared to controls.

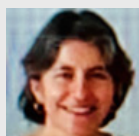
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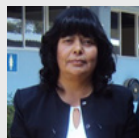
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