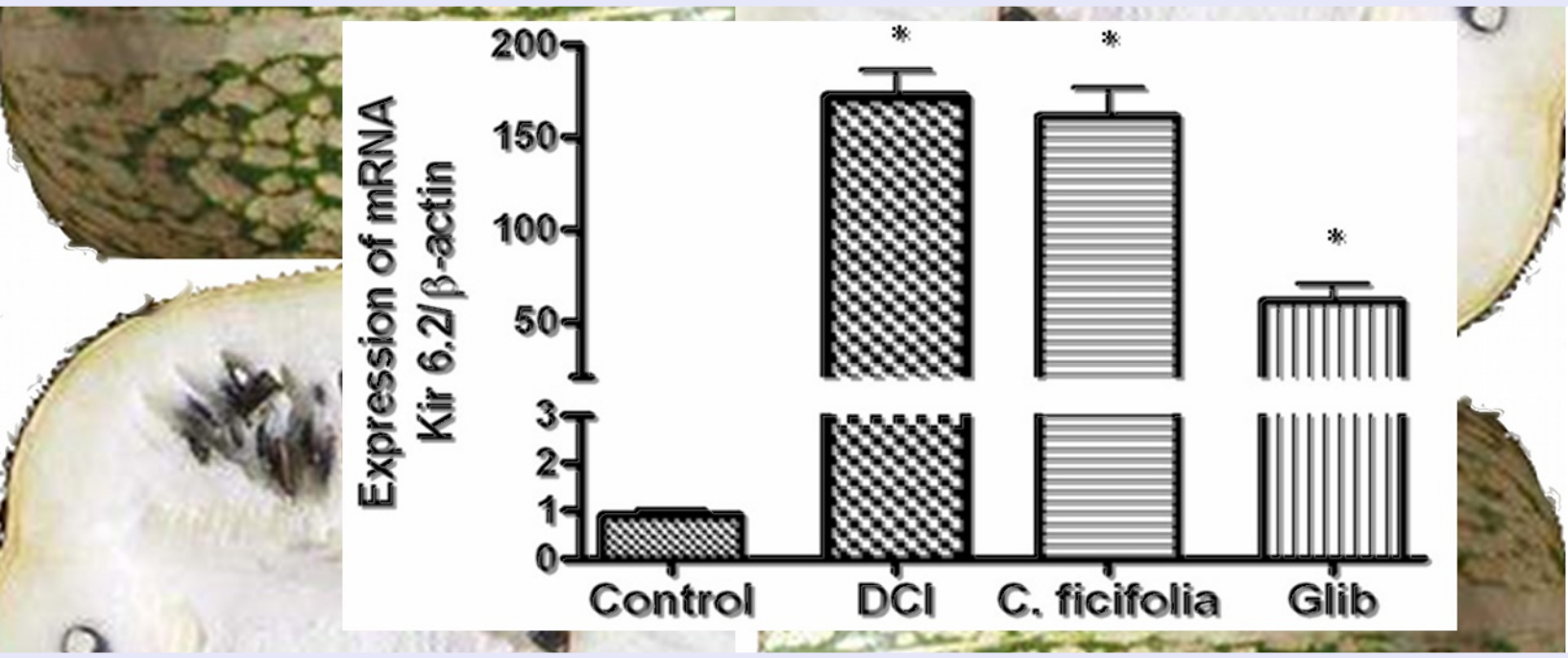
Number 3 Issue 1 February 2013

International Biotechnology Color Journal

A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY
and Covering Its Many Hues, Tints, Tones & Shades



Regular issue:
Cucurbita ficifolia Bouché fruit as insulin secretagoguee.

Produced and hosted by *Centro de Investigación Científica de Yucatán, A.C.*,
in collaboration with *Universidad Nacional Autónoma de México*,
and the *International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society*
Int. biotechnol. color j., ISSN 2226-0404

Number 3 Issue 1 February 2013

full text provided by www.ibcj.org.mx

International Biotechnology Color Journal

A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY and Covering Its Many Hues, Tints, Tones & Shades

The International Biotechnology Color Journal appears every 4 months in the last week of February, June and October of each year, starting in October 2011. It is a non-profitable Electronic Publication. In its initial phase it does not requires neither publication nor access fees. Its goal is the publication of Original Scientific Information, previously unpublished, and provided by the contributing authors out of its own will. *Int. biotechnol. color j., ISSN 2226-0404.*

The INTERNATIONAL BIOTECHNOLOGY COLOR JOURNAL

(IBCJ)

is the official quarterly publication of the **International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society A.C.** (IFBR&ESCHNSAST&S).

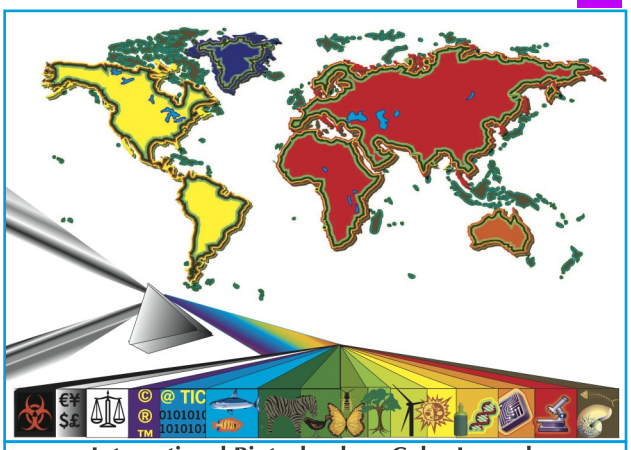
The IFBR&ESCHNSAST&S is a civil association and nonprofit organization.

Its main goal is to help bringing together efficient biotechnological solutions for many human problems, with the need for environmentally friendly and sustainable processes.

As means to such goal, the Foundation counts on education to create social awareness to biotechnology's benefits and risks, and to promote the formation of highly qualified professionals and research scientists.

The constitutive act of the foundation was signed in the Heroic City of *Huajuapan de León, Oaxaca*, on September 14th, 2009, at the *NOTARÍA PÚBLICA No. 61 de los ESTADOS UNIDOS MEXICANOS*. As .

Susana Lozano Muñiz
President of the Foundation



International Biotechnology Color Journal

The International Biotechnology Color Journal is produced and Hosted by Centro de Investigación Científica de Yucatán, A.C., Calle 43 No. 130, Colonia Chuburná de Hidalgo. Mérida Yuc., Mexico. Tel: (+52) 999 942 8330. Fax: (+52) 999 942 3900. RFC. CIC791116770.
Director: Rogelio Rodríguez Sotres, Faculty of Chemistry, Universidad Nacional Autónoma de México. Cd. Universitaria. Ave. Universidad 3000, C.P. 04510, Coyoacán, D.F. México. E-mail: *sotres@unam.mx*
The staff does not earn a salary or receive any monetary compensation for the activities associated to the production of this Journal.

Number 3 Issue 1 February 2013

full text provided by www.ibcj.org.mx

International Biotechnology Color Journal

A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY and Covering Its Many Hues, Tints, Tones & Shades

Editorial section

International Biotechnology Color Journal (IBCJ) is an electronic Open Access journal, devoted to the publication of peer-reviewed articles covering all the fields of biotechnology.

With this number we celebrate our first year of publication with main focus on the dissemittion of scientific peer reviwed papers. In addition ICBJ intent is to provide a forum for reviews of special interest, notes presenting relevant findings in a short format, essays with new technical advances or relevant updates of reported protocols, book reviews, scientific meetings, and letters to the editor. The present issue offers a sample of such diversity.

Instructions for every type of contribution are presented in the journal's Homepage and at the end of the present issue. The Editorial Board of IBCJ is fully committed to publish novel contributions in all areas of biotechnology. Submissions are reviewed from a rigorous optic of scientific criticism and all original contributions within the scope of the journal are welcomed.

If you are interested in participating as reviewer, please send us a letter, and a CV stressing your experience in the filed. Send this information by e-mail to "Dr. Jose Juan Zúñiga Aguilar" <zuniga@cicy.mx>

Editorial comments to the contents of this issue

By José Juan Zúñiga-Aguilar, Chief editor.

In this issue, a report of Miranda-Pérez *et al*. offers an interesting proposal on the mechanism hypoglycemic activity effects by extracts of *Cucurbita ficifolia* (chilacayote), an empiric effect largely accepted by the traditional knowledge. Using cultures of the insulin-producing RINm5F cells, they found that crude extracts of the plant, as well as samples of the D-chloro-inositol control treatment, promoted the increment of transcripts encoding insulin and Kir6.2 channels, respectively. This result offers relevant information supporting the traditional use of this plant as an alternative treatment of the type 2 Diabetes mellitus disease (T2D). Being T2D a public health problem in Mexico, the use of better-accepted drugs for its treatment constitutes an alternative to deal with a problem of high impact in health, economy and social development, and which is increasing dramatically in Mexico.

Number 3 Issue 1 February 2013

full text provided by www.ibcj.org.mx

International Biotechnology Color Journal

A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY and Covering Its Many Hues, Tints, Tones & Shades

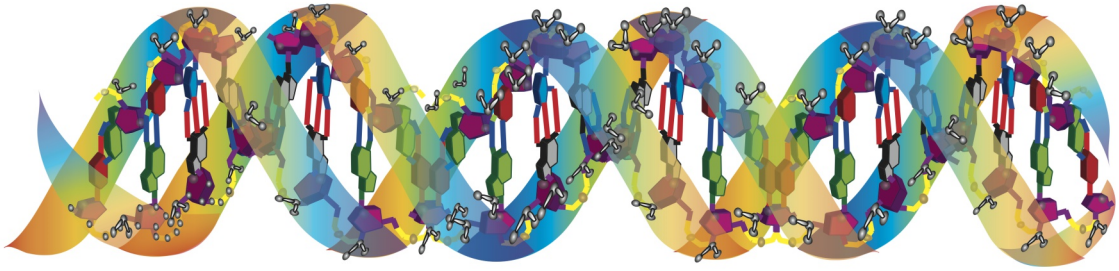
Table of Contents

Number 3 Issue 1 February 2013

full text provided by www.ibcj.org.mx

International Biotechnology Color Journal

A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY and Covering Its Many Hues, Tints, Tones & Shades



Regular Issue, February 2013

Number 3 Issue 1 February 2013

full text provided by www.ibcj.org.mx

International Biotechnology Color Journal

A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY and Covering Its Many Hues, Tints, Tones & Shades

Why to publish on IBCJ?

By Rogelio Rodríguez-Sotres

Dear reader,

As the size of world's population increases, so does the scientific community. At the same time, technological aids arise in all areas and open the door to new opportunities. The editorials handling scientific communications are not an exception to this trend, and many new editorial houses have emerged.

Most of these new editorials operate under the Open Access Publishing model, commonly known as "author pays", contrary to the traditional publishing model where the reader covers the cost through his/her subscription fee.

The Open Access Model has grown rapidly because:

A) Lowers production costs
B) Increases the paper's visibility
C) Reduces "piracy" problems
D) It is considered more ethical, as the author is asked to endorse this work intellectual's ownership to the editorial, and the editorial should not be profiting with it. In the Open Access Publishing model the author pays for editorial services and information backup.

Unfortunately, as with any busyness, ethical misconduct happens, and some editorial may try to deceive authors setting up sites with all sorts of misleading data. These predatory editorials scam authors into sending their manuscripts with counterfeit invitations, and almost always, the contributors are not warned of the publications charges, until an invoice is issued.

There are very good editorials operating under the "author pays" model, and in fact, some of those editorials have created and published their own ethical guidelines, somehow setting a standard for the industry.

WHAT WE CAN OFFER:

A) We take our work seriously. The main staff of IBCJ are scientist, we understand the problems of the scientist, and specially those in the Latin America Scientific community, but we know scientist do not want their work to go published unreviewed and poorly edited.

B) Therefore, all papers are peer-reviewed, no matter if the author is one of our own editors or staff. In this last case, we make everything in our reach to avoid conflicts of interest, through a careful choice of editor and reviewers. If you have doubts, please contact authors of papers already published. Contact addresses and e-mails are included on every article. Nobody can rate our work better than those who have already used it. Please read the papers published, judge their quality by yourself.

C) We have a recognized set of Editors, and we are trying the get even better in this aspect.

D) The Journal's style is appealing and production has high standards. Just look at the articles.

E) We do this out of our own convictions, not for money. None of the staff members get any monetary compensation for his/her effort. we have no reason to publish your work if it has no value.

F) We have manage to cover all expenses with no charges to authors or readers! We are currently seeking funding to pay for DOI numbers, and be granted indexation on international databases, but are committed to keep publication costs at their minimum.

WHAT WE ARE NOT:

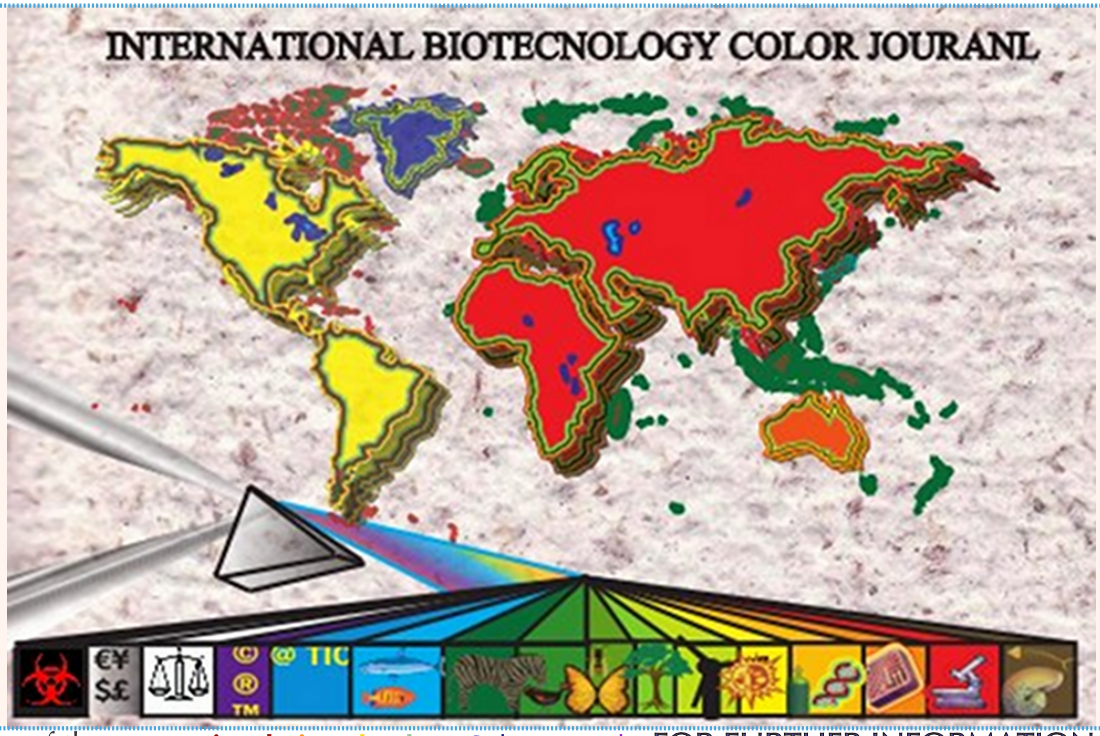
A) We are not trying to deceive you, currently we do not have DOI numbers

B) We are not presently indexed on scientific databases other the Google Scholar and Scopus, which offer wide coverage, with little discrimination.

C) We still publish a few articles per year.

PLEASE FEEL FREE TO WRITE US. LET US KNOW YOUR SUGGESTIONS AND COMMENTS TO:

<sotres@unam.mx>



From the office of the **International Biotechnology Color Journal** - FOR FURTHER INFORMATION CONTACT:

Rogelio Rodríguez-Sotres, Faculty of Chemistry, Universidad Nacional Autónoma de México

Tel (+52)5556225285; Fax (+52)5556225329; e-mail: *sotres@unam.mx*

International Biotechnology Color Journal

Cucurbita ficifolia Bouché fruit acts as an insulin secretagogue in RINm5F cells

María Elizabeth Miranda-Pérez[1], María Del Carmen Escobar-Villanueva[2], Clara Ortega-Camarillo[3], Fausto Sánchez-Muñoz[4], Julio César Almanza-Pérez[2], Francisco Javier Alarcón-Aguilar[2*].

[1]Posgrado en Biología Experimental. Universidad Autónoma Metropolitana (UAM).

[2] Laboratorio de Farmacología. Depto. Ciencias de la Salud, Universidad Autónoma Metropolitana (UAM)

[3] UIM en Bioquímica. Hospital de Especialidades CMN SXXI. Instituto Mexicano del Seguro Social.

[4] Instituto Nacional de Cardiología "Ignacio Chávez" Depto. Inmunología

Chief Editor: Dr. José Juan Zúñiga Aguilar.
Received November 15th, 2012. Revised form February, 20th. Accepted February 28th.

Abstract

Diabetes mellitus (DM) is an important global public health problem. Despite the availability of drug therapy, patients have continued using plants with anti-diabetic properties as alternative treatments for diabetes. Cucurbita ficifolia Bouché (C. ficifolia) is a plant cultivated in Mexico for its edible fruit that is medicinally used to control type 2 DM (T2D). The hypoglycemic effect of C. ficifolia has been demonstrated in different experimental models and in T2D patients. However, no studies have determined the mechanism of action of this hypoglycemic effect. The aim of the present investigation was to determine if the hypoglycemic action of C. ficifolia and D-chiro-Inositol (DCI; a compound found in the fruit of C. ficifolia) occurs through an increase in the production of insulin. An aqueous extract of this fruit was obtained and standardized by its content of DCI, the principal hypoglycemic compound of the fruit. To study the mechanism of hypoglycemic activity of this extract and DCI alone, RINmF5 cells were exposed to different concentrations of both the extract and DCI, and the production of insulin and Kir6.2 channels were measured. The mRNA expression levels of insulin and Kir6.2 were found to be increased in cells that were treated with DCI and the C. ficifolia aqueous extract. This effect suggests a mechanism of action that involves both the expression and secretion of insulin. This research maintains the interest in developing new nutraceutics from C. ficifolia fruits for use in the treatment of T2D.

*To whom all correspondence should be addressed. 186. S. Rafael Atlixco, Col. Vicentina. Iztapalapa, P.O. 09340., México D.F.
E-mail: aaasj2@prodigy.net.mx
Tel.: +52 55 58046483. Fax: +52 55 58044727

Keywords

Type 2 diabetes, insulin, Kir6.2, Cucurbita ficifolia Bouché, D-chiro-inositol.

Abbreviations

DM, diabetes mellitus; T2D, type 2 diabetes mellitus; SUR1, sulfonylurea receptor 1; DCI, D-chiro-inositol;

YELLOW: Nutritional Biotechnology

Introduction

Type 2 diabetes mellitus (T2D) is a set of metabolic disorders characterized by increased levels of blood glucose (hyperglycemia) due to defects in insulin production and secretion by the pancreatic β cells (1-4).

One of the key factors in the secretion of insulin by pancreatic β cells is the ATP-dependent K^+ channels (K^+_{ATP}). These channels are formed by two tetramers of the subfamily Kir6.2 and the sulfonylurea receptor 1 (SUR1) (5). Both Kir6.2 and SUR1 have the ability to induce changes in membrane electrical excitability. This change leads to calcium channel opening and an increase in cytoplasmic calcium concentration in beta cells, which triggers the mobilization and release of insulin (6). Sulfonylureas are anti-diabetic agents that act by releasing endogenous insulin following binding to SUR1 (7,8).

Despite the widespread use of antidiabetic drugs, more than 70% of the world population uses medicinal plants as the only available alternative for the treatment of their diabetes-related health problems (9). Furthermore, many medicinal plants have been reported to have "anti-diabetic" properties (10-12). Cucurbita ficifolia Bouché (C. ficifolia, Cucurbitaceae) is cultivated in Mexico for its edible fruit (13). It is an annual monoecious plant, which is grown primarily in the states of Mexico, Hidalgo, Puebla and Veracruz. C. ficifolia is commonly known as "chilacayote", and its immature fruit is used for preparing different dishes. The mature fruit of C. ficifolia is used to make a traditional candy (14). In the states of Mexico and Morelos, the annual production of C. ficifolia is 4,706 tons and the harvested area is 300 hectares (15).

C. ficifolia fruits are also medicinally used to control type 2 DM (T2D) and their hypoglycemic effect has been demonstrated in different experimental models and in T2D patients. D-chiro-inositol (DCI) has been proposed to be the active component in C. ficifolia (16). DCI, originally discovered as a mediator of intracellular insulin action, has been shown to accelerate the dephosphorylation of the glycogen synthase and pyruvate dehydrogenase, two regulatory enzymes of the glucose oxidation pathways (14,17). Clinical studies have demonstrated a linear relationship between body DCI deficiency and the degree of insulin resistance in subjects with T2D. In addition, the administration of DCI to diabetic rats and rhesus monkeys improved glucose utilization and insulin action (17). In streptozotocin-induced diabetic rats, daily administration of C. ficifolia fruit and DCI over a 30-day period reduced glycemia and increased the levels of liver glycogen, total hemoglobin and insulin (16). Although both C. ficifolia fruit extracts and DCI might be used as coadjutants in DM control (18,19), there are no studies that explain the mechanisms of action involved in their hypoglycemic effect. This is very important because the juice of the fruit has been studied in Mexico as a hypoglycemic agent in experimental animal models and in diabetic patients (19). Therefore, the aim of this work was to determine if the hypoglycemic effect of the C. ficifolia fruit extracts and DCI may be explained by a rise in the expression levels of insulin and Kir6.2 in RINmF5 cells.

Material and methods

Plant material

Fresh mature fruits of C. ficifolia with a diameter of 18–20 cm and an approximate weight of 4 kg were gathered in the Acolman municipality, State of Mexico during April of 2011. The endocarp, free of seeds, was cut into thin slices and placed in a container at room temperature with constant aeration for dehydration. The dried material was ground in an electrical Wiley mill, using a grid of 1 mm diameter (14). This material (100 g) was macerated with water (1 L) for 72 h in a laminar flow hood. The aqueous phase was filtered and centrifuged at 805 × g to obtain a precipitate, which was separated and freeze-dried (LABCONCO) (yield = 5%) (19). Quantification of the DCI content in the C. ficifolia extract was performed using high performance liquid chromatography (HPLC Waters 2695 separation module) as reported by Roman-Ramos et al. (2012) (14). This resulted in an extract with a concentration of 3.3 mg of DCI/g of extract. A pure DCI standard was obtained from Sigma-Aldrich.

RINm5F cell culture

RINm5F, which is an insulin-producing cell line derived from a pancreatic islet tumor, cells were commercially acquired from the American Type Culture Collection (ATCC). RINm5F cells were grown in a monolayer culture using RPMI 1640 medium (11.1 mM glucose) (GIBCO™), supplemented with 10% fetal bovine serum (ATCC), 2 mM 1–glutamine, 1 mM sodium pyruvate and 2 μg L^{-1} of gentamycin (Invitrogen). The cells were grown at 37 °C in disposable plastic bottles (Nunc™) and in a humidified atmosphere of 5% CO_2/95% air (20–22). The medium was replaced twice a week.

MTT assay

Cell viability, following treatment with both the extract of C. ficifolia fruit and DCI alone, was measured using the 3–(4,5–dimethylthiazole–2–yl)–2,5–dihenyltetrazolium bromide (MTT, sigma) assay, according to Mosmann (23). The assay measures the conversion of MTT to insoluble

formazan by the dehydrogenase enzyme activity of the intact cells; this assay is widely used for studies of cytotoxicity, viability and proliferation. The amount of the formazan produced is directly proportional to the number of living cells (22). The RINm5F cells were seeded into 96-well microplates at a semi-confluent density (5000 cells/well). After 24 h, the medium was replaced with complete medium containing concentrations of DCI ranging from 0.1 to 50 µM in the aqueous extract of C. ficifolia fruit or the equivalent concentrations of DCI alone (Sigma). The cells were treated for 24 h. They were then washed with phosphate-buffered saline (PBS), pH 7.4, and a solution of 0.1 mg mL^{-1} MTT in PBS (pH 7.5) was added. The cells were incubated for 3 h at 37 °C, followed by washing with PBS. Then, 200 µL of 40 mM HCl (prepared in isopropanol) was added to each well for 15 min to solubilize the produced formazan. The OD was read at 570 nm (23). The data were expressed as the percentage of viable cells following treatment with the aqueous extract of C. ficifolia and DCI compared to the control cells.

Because the treatments with the aqueous extract of C. ficifolia fruit were based on its DCI content, we chose the equivalent concentrations for the DCI alone treatments of the RINm5f cells and quantify the insulin and Kir6.2 mRNA expression levels. Thus, the choice of the concentrations to treat the cells with the aqueous extract of C. ficifolia fruit and with DCI alone (0.25 µM of DCI in both cases) preceded the extraction of total RNA from the treated cells.

RNA isolation, reverse transcriptase polymerase chain reaction and real time PCR

For the extraction of total RNA, cells were seeded at 1 x 10^6 cells (RINm5f) per treatment in 6–well culture dishes and incubated for 24 h. Both the aqueous extract of C. ficifolia fruit and the DCI treatments were performed at a concentration of 0.25 µM and over 24 h. After treatment, the cells were washed with 1 mL ice-cold PBS and solubilized with 1 mL of TRIzol® reagent (Invitrogen). RNA was treated with chloroform, centrifuged at 12,235 × g for 15 min at 4 °C and precipitated with ethanol as described by Chomczynski (21, 25). RNA was extracted and re-dissolved in diethylpyrocarbonate treated water and the OD at 260 nm was measured to determine the RNA concentration. cDNA was synthesized using 2 µg total RNA by reverse transcriptase PCR using the ImProm II reverse transcription system (Promega). The cDNA was amplified by an enzyme DNA polymerase kit, the DNA master plus SYBR Green 1 PCR MasterMix (Applied Biosystems) (20) for the following genes: Insulin NM_09129.3 (5′-TGCCCAGGCTTTTGTCAAAC-3′), Kir6.2 NM_031358.3 (5′-GTACAGATCATTGTGGGCGT-3′) and β–actin NM_031144.3 (5′-GTGGGTATGGGTCAGAAGGA-3′), which was used as a control.

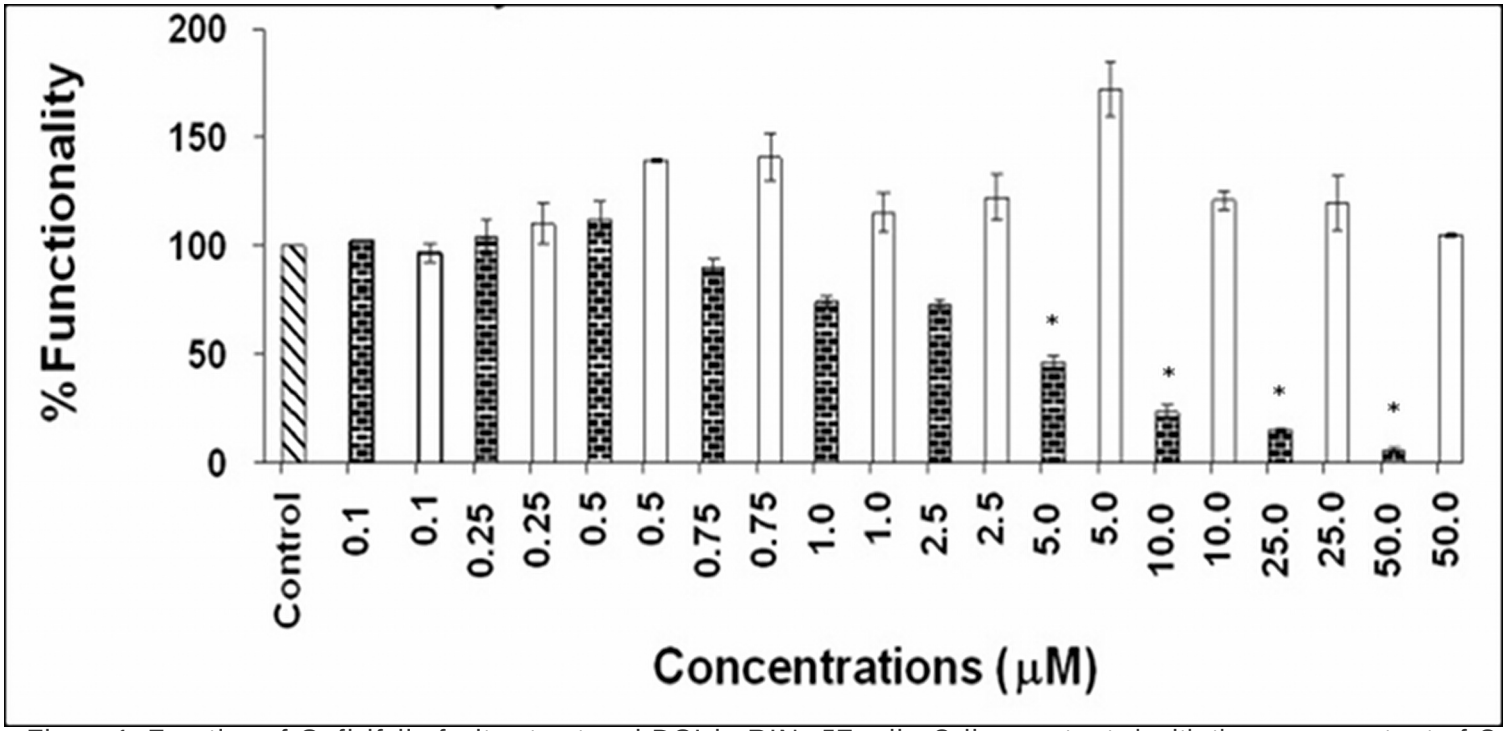


Figure 1. Function of C. ficifolia fruit extract and DCI in RINm5F cells. Cells were treated with the aqueous extract of C. ficifolia fruit and DCI at concentrations ranging from 0.1 to 50 µM (x-axis). The Y-axis shows the percentage of activity in the treated cells. A concentration of 0.25 µM was chosen for treating the cells (n=6). *Significantly different compared to control ($p<0.05$).

Statistical analysis

Data are presented as the mean ± standard error of the mean (S.E.M.). Significant differences among the treatments were determined by an analysis of variance using the Tukey–Kramer Multiple Comparison post–hoc test ($p<0.05$).

Results and Discussion

Fig. 1 shows the percentage of dehydrogenase functionality in the RINm5f cells treated with different concentrations of both the aqueous extract of C. ficifolia fruit and DCI. At a concentration of 1 µM of DCI in the aqueous extract of C. ficifolia fruit, a 20% cell functionality decrease compared to the control group ($p<0.05$) was observed; this effect was indicative of a concentration-dependent cytotoxic effect. A DCI alone concentration of 0.25 µM showed a retention of 98% of the dehydrogenase functionality of the cell compared to the control group. DCI alone did not show a cytotoxic effect at any of the concentrations examined. Therefore, a concentration of 0.25 µM DCI was chosen to examine insulin and Kir6.2 gene expression following treatment with the aqueous extract of C. ficifolia fruit and DCI alone.

A statistically significant increase in insulin mRNA expression (a 5-fold increase) compared with the control group was observed after treatment with the aqueous extract of C. ficifolia fruit and with DCI alone in the RINm5F cells (Figure 2). Therefore, the hypoglycemic effect of C. ficifolia may be explained by an increase in the expression of the insulin gene. Various studies that focused on gene therapy for diabetes have proposed the use of agents with a strong relationship between the stimulation of insulin gene expression and its secretion (26). Ultra-structural analysis of beta cells has shown reduced insulin granules in human D2T. These survival and functional changes are accompanied by modifications of beta cells gene and protein expression (27). Finding agents that regulate insulin gene expression to give an advantage to beta cells holds considerable promise and could provide the beta cells the necessary flexibility to adapt to the changing environment in DT2. If C. ficifolia exerts any effect on the regulation of gene expression of insulin, it would be a viable therapeutic agent for the treatment of T2D.

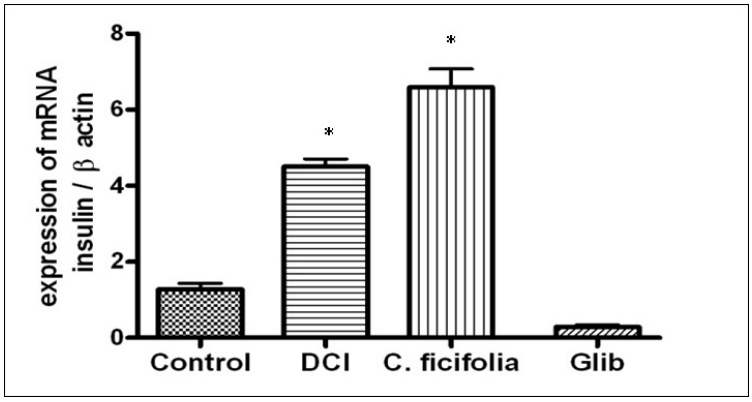


Figure 2. Insulin mRNA gene expression in RINm5F cells. Cells were treated with the aqueous extract of C. ficifolia fruit and DCI at a concentration of 0.25 µM for 24 h. The aqueous extract of C. ficifolia fruit and DCI induced insulin gene expression, which was increased 5-fold (n=6). *Significantly different against control ($p<0.05$).

Studies in rat beta cells have associated the effect of glybenclamide on insulin mRNA expression with an increase in the cytoplasmic concentration of $[Ca^{2+}]$ after 24 h treatment (28). Therefore, the aqueous extract of C. ficifolia fruit could stimulate insulin gene expression through an increase in cytoplasm $[Ca^{2+}]$. Although our results are in agreement with other studies in which C. ficifolia fruit extracts and DCI have shown an effect on insulin secretion (16), other studies are required that measure insulin secretion and the cytoplasmic concentration of $[Ca^{2+}]$. Nevertheless, in our studies, glybenclamide (24 h, 4 µM) did not cause an increase in the expression of insulin mRNA as has been reported in previous studies (28). This is likely due to the difference in the treatment concentrations (24 h, 0.5 µM).

ATP-sensitive potassium channels (K^+_{ATP}) in pancreatic beta cells contain SUR1 and the inwardly rectifying potassium channel (Kir) 6.2 subunits. SUR1 and the Kir6.2 protein assemble into a potassium channel and play a key role in the regulation of insulin secretion (29). We evaluated the effect of the aqueous extract of C. ficifolia fruit and DCI on Kir6.2 mRNA expression. Figure 3 shows that in RINm5F cells, the aqueous extract of C. ficifolia fruit and DCI significantly increased Kir6.2 mRNA expression (greater than 100-fold) compared with the control group. This is the first report of the effect of DCI and the aqueous extract of C. ficifolia fruit on Kir6.2 mRNA expression. The K^+_{ATP} channel plays an important role in insulin secretion, and this investigation opens the possibility that in the hypoglycemic effect of DCI and C. ficifolia is involved the expression of both insulin and DCI. In other studies, the expression of Kir6.2 has been positively correlated with cellular protein content (30). Thus, the increase in Kir6.2 mRNA by the aqueous extract of C. ficifolia fruit and DCI should indicate an increase in the protein content, which would be crucial for the secretion of insulin. Other studies are required to confirm the association among Kir6.2 expression, protein content and insulin secretion.

Notably, the mechanism of action of glibenclamide involves a block of the K^+_{ATP} channel by binding to SUR1 in

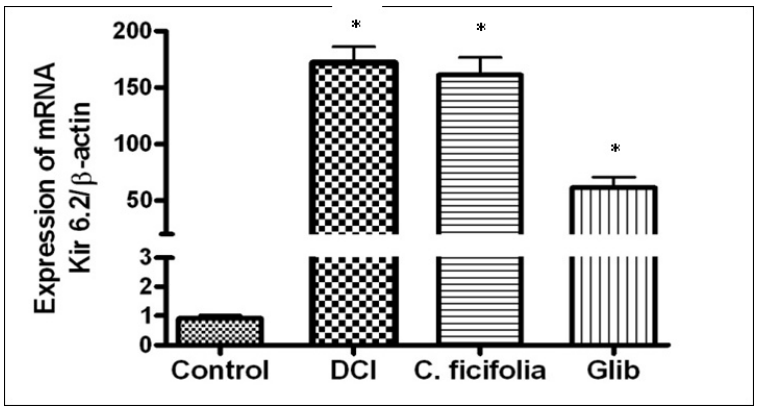


Figure 3. Kir 6.2 mRNA gene expression in RINm5F cells. Cells were treated with the aqueous extract of C. ficifolia fruit and DCI at a concentration of 0.25 µM for 24 h. The aqueous extract of C. ficifolia fruit and DCI induced Kir6.2 gene expression, which was increased 100–fold (n=6). *Significantly different against control ($p<0.05$).

beta cells. This depolarizes the cell plasma membrane, inducing the opening of [Ca^{2+}] channels and the secretion of insulin (31). In this study, glibenclamide stimulated the expression of Kir6.2 without affecting insulin expression (Figure 3). However, the C. ficifolia fruit extract and DCI increased the mRNA expression of both insulin and Kir6.2; therefore, the C. ficifolia fruit extract and DCI must have a different hypoglycemia inducing mechanism than glibenclamide. This is likely the result of distinct chemical components in the C. ficifolia fruit extract other than DCI.

Previous phytochemical studies have shown the presence of active principles in the aqueous extracts of C. ficifolia fruit (32). The aqueous extract contains DCI (3.3 mg/g of extract), and it has been reported to contain flavonoids and cucurbitacins. There have been similar findings in other Cucurbitacea species (33). Flavonoids are secondary metabolites of plants, and have been characterized as being soluble in water. Flavonoids have been principally associated with increased in insulin secretion (34-35). Flavonoids are likely to be associated with the expression of insulin mRNA, which clearly agrees with the greater effect of the aqueous extract of the C. ficifolia fruit compared to DCI alone.

The fruit of C. ficifolia has been associated with a hypoglycemic effect, and it has been shown to have additional benefits as an antioxidant and an anti-inflammatory (10,14,19). It is probable that these actions may be attributed components in the extract other than DCI, such as, flavonoids (36) or curubitacines (37). However, a complete characterization of the aqueous extract is necessary to correlate these effects.

C. ficifolia has been reported as a useful alternative for the treatment of T2D (10-12,14). The activity of C. ficifolia and DCI on the mRNA expression levels of proteins implicated in the production and secretion of insulin in pancreatic beta cells represents a notable finding. Other studies have focused on verifying the hypoglycemic effect of C. ficifolia in both diabetic patients and animal models (16,18-19). These studies have provided a great contribution to the study of medicinal plants. However, this study represents the first attempt to elucidate the mechanism of action of C. ficifolia: an increase in the expression of insulin and K^+_{ATP} channels, which is likely due to DCI.

Several nutraceuticals used in clinical practice have been shown to target the pathogenesis of diabetes mellitus (38). In our laboratory, a jelly developed from pomegranate juice with the addition of a pomegranate rind extract, has the potential to control DM (39). Therefore, in the same manner, we can develop a nutraceutical from C. ficifolia.

Conclusion

The aqueous extract of C. ficifolia fruit and DCI increased mRNA expression for insulin and Kir6.2 in RINm5F cells. These results suggest a mechanism of action involving the stimulation of insulin and Kir6.2 (a protein widely involved in the secretion of insulin) production, which represents a useful alternative for DM control. Therefore, the fruits of C. ficifolia may be considered to be a potential source of raw material for obtaining new oral hypoglycemic drugs and for the development of a new nutraceutic for DM control.

Acknowledgements

We thank CONACYT for the grants awarded to M.B.E Maria Elizabeth Miranda Perez. This research was partially supported by PROMEP-SEP (P/CA-15-2006-35-53). We appreciate the support of Dr. Maria Concepcion Gutierrez Ruiz, from the Laboratory of Cellular Physiology. The authors would also like to acknowledge Biol. Gerardo Garcia Velazquez, MSc Jesus Vladimir Hernandez-Rosado and MSc Jessica Garcia Gonzalez from the Postgrad in Experimental Biology at Universidad Autónoma Metropolitana, for their technical assistance.

References

1. American Diabetes Association (2011) Standards of medical care in diabetes. Diabetes Care. 34 Suppl 1:S11-61.

2. Ma Za (2012) The role of peroxidation of mitochondrial membrane phospholipids in pancreatic β–cell failure. Curr Diabetes Rev 8:69–75

3. Villalpando S, Shamah-Levy T, Rojas R, Aguilar-Salinas CA (2010) Trends for type 2 diabetes and other cardiovascular risk factors in Mexico from 1993-2006. Salud Publica Mex 52 Suppl 1:S72–79

4. Wild S, Roglic G, Green A, Sicree R, King H (2004) Global prevalence of diabetes estimates for the year 2000 and projections for 2030. Diabetes Care 27:1047–1053

5. Yamazaki H, Zawalich K, Zawalich W (2010) Physiologic implications of phosphoinositides and phospholipase C in the regulation of insulin secretion. J Nutr Sci Vitaminol 56:1–8

6. Florez J, Armijo JA, MediaVilla A (1997) Farmacologia Humana, 3th Edition, Chapter 54, Ed. Masson, S.A. Mexico. pp. 927–943

7. Henquin JC (2000) Pathways in β-cell stimulus-secretion coupling as targets for therapeutic insulin secretagogues. Diabetes 49:1751–1760

8. Willenborg M, Hatlapatka K, Ghaly H, Belz M, Panten U, Rustenbeck I (2011) Studies of first phase insulin secretion using imposed plasma membrane depolarization. Front Biosci (Schol Ed) 1:662–679

9. Farnsworth NR, Marles RJ (1995) Antidiabetic plants and their active constituents. Phytomedicine 2:137–189

10. Romero CO, Reyes MH, Aguilar SL, Huerta RM, Tortoriello GJ (2009) Use of medicinal plants among patients with diabetes mellitus type 2 in Morelos, Mexico, BLACPMA 8:380–388

11. Prabhakar PK, Doble M (2008) A target based therapeutic approach towards diabetes mellitus using medicinal plants. Curr Diabetes Rev. 4(4):291–308

12. Qi LW, Liu EH, Chu C, Peng YB, Cai HX, Li P (2010) Anti-diabetic agents from natural products--an update from 2004 to 2009. Curr Top Med Chem. 10(4):434–457

13. Seshadri, VS, More TA (2009) Cucurbit vegetables: biology, production and utilization. Studium Press Pvt. Limited, India, pp. 482

14. Roman-Ramos R, Almanza-Perez JC, Fortis-Barrera A, Angeles-Mejia S, Banderas-Dorantes TR, Zamilpa-Alvarez A, Diaz-Flores M, Jasso I, Blancas-Flores G, Gomez J, Alarcon-Aguilar FJ (2012) Antioxidant and anti-inflammatory effects of a hypoglycemic fraction from Cucurbita ficifolia Bouche in streptozotocin-induced diabetes mice. Am J Chin Med 40:97–110

15. Molina MJ, Leobigildo CT (2006) Informe Nacional sobre el estado de los recursos fitogenéticos para la agricultura y la alimentación. Cuadro 1.1 Importancia económica de las especies autóctonas anuales cultivadas en México. Año 2003. Sociedad Mexicana de fitogenética , A. C., SOMEFI. México. pp. 19

16. Xia T, Wang Q (2006) D-chiro-inositol found in Cucurbita ficifolia (Cucurbitaceae) fruit extracts plays the hypoglycaemic role in streptozocin-diabetic rats. J Pharm Pharmacol 58:1527–1532

17. Larner J (2002) D-Chiro-Inositol - Its functional role in insulin action and its deficit in insulin resistance. Experimental Diab 3:47–60

18. Acosta JL, Jimenez E, Juarez MA, Diaz JC (2001) Hypoglycemic action of Cucurbita ficifolia on type 2 diabetic patients with moderately high blood glucose levels. J Ethnopharmacol 77:99–101

19. Alarcon-Aguilar FJ, Hernandez-Galicia E, Campos-Sepulveda AE, Xolalpa-Molina S, Rivas-Vilchis JF, Vazquez-Carrillo LI, Roman-Ramos R (2002) Evaluation of the hypoglycemic effect of Cucurbita ficifolia Bouché (Cucurbitaceae) in different experimental models. J Ethnopharmacol 82:185–189

20. Chen F, Zhu Y, Tang X, Sun Y, Jia W, Sun Y, Han X (2011) Dynamic regulation of PDX-1 and FoxO1 expression by FoxA2 in dexamethasone-induced pancreatic β–cells dysfunction. Endocrinology. 5:1779–1788

21. Numazawa S, Takase M, Ahiko T, Ishii M, Shimizu S, Yoshida T (2012) Possible involvement of transient receptor potential channels in electrophile-induced insulin secretion from RINm5F cells. Biol Pharm Bull. 35:346–354

22. Lim S, Rashid MA, Jang M, Kim Y, Won H, Lee J, Woo JT, Kim YS, Murphy MP, Ali L, Ha J, Kim SS (2011) Mitochondria-targeted antioxidants protect pancreatic β–cells against oxidative stress and improve insulin secretion in glucotoxicity and glucolipotoxicity. Cell Physiol Biochem. 28:873–886

23. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay. J Inmunol Methods 65:55–63

24. Stockert JC, Blázquez-Castro A, Cañete M, Horobin RW, Villanueva A (2012) MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. Acta Histochem. 114:785–796

25. Chomczynski P.A (1993) Reagent for the single step simultaneous isolation of RNA, DNA and proteins from cells and tissue samples. Biotechniques 15:532-534, 536–537

26. Ahmad Z, Rasouli M, Azman AZ, Omar AR (2012) Evaluation of insulin expression and secretion in genetically engineered gut K and L–cells. doi: 10.1186/1472-6750-12–64

27. Marchetti P, Bugliani M, Boggi U, Masini M, Marselli L (2012) The pancreatic beta cells in human type 2 diabetes. Adv Exp Med Biol. 771:288–309

28. Ling Z, Wang Q, Stangé G, In't Veld P, Pipeleers D (2006) Glibenclamide treatment recruits beta-cell subpopulation into elevated and sustained basal insulin synthetic activity. Diabetes. 55(1):78–85

29. Mikhailov MV, Mikhailova EA, Ashcroft SJ (2000) Investigation of the molecular assembly of beta-cell K(ATP) channels. FEBS Lett. 482:59–64

30. Raeis-Dauvé V, Philip-Couderc P, Faggian G, Tessari M, Roatti A, Milano AD, Bochaton-Piallat ML, Baertschi AJ (2012) Increased expression of adenosine triphosphate-sensitive K^+ channels in mitral dysfunction: mechanically stimulated transcription and hypoxia–induced protein stability? J Am Coll Cardiol. 59:390–396

31. Kim JY, Lim DM, Park HS, Moon CI, Choi KJ, Lee SK, Baik HW, Park KY, Kim BJ (2012) Exendin-4 protects against sulfonylurea-induced β–cell apoptosis. J Pharmacol Sci. 118:65–74

32. Andrade-Cetto A, Heinrich M (2005) Mexican plants with hypoglycaemic effect used in the treatment of diabetes. J Ethnopharmacol 99:325–348

33. Chen JC, Chiu MH, Nie RL, Cordell GA, Qiu SX (2005) Cucurbitacins and cucurbitane glycosides: structures and biological activities. Nat Prod Rep. 22:386–399

34. Ansarullah, Bharucha B, Dwivedi M, Laddha NC, Begum N, Hardikar AA, Ramachandran AV (2011) Antioxidant rich flavonoids from Oreocnide integrifolia enhance glucose uptake and insulin secretion and protects pancreatic β-cells from streptozotocin insult. BMC Complementary and Alternative Medicine. 11:1–13

35. Matsuda T, Ferreri K, Todorov I, Kuroda Y, Smith CV, Kandeel F, Mullen Y (2005) Silymarin protects pancreatic beta-cells against cytokine-mediated toxicity: implication of c-Jun NH_2–terminal kinase and janus kinase/signal transducer and activator of

transcription pathways. Endocrinology. 146:175–185

36. Ibarra-Alvarado C, Rojas A, Mendoza S, Bah M, Gutiérrez DM, Hernández-Sandoval L, Martínez M (2010) Vasoactive and antioxidant activities of plants used in Mexican traditional medicine for the treatment of cardiovascular diseases. Pharm Biol. 48:732–739
37. Chen X, Bao J, Guo J, Ding Q, Lu J, Huang M, Wang Y (2012) Biological activities and potential molecular targets of cucurbitacins: a focus on cancer. Anticancer Drugs. 23:777–787
38. Davì G, Santilli F, Patrono C (2010) Nutraceuticals in diabetes and metabolic syndrome. Cardiovasc Ther. 28:216–226
39. Ventura-Sobrevilla J M (2012) Elaboración de una galea adicionada con fitoquímicos de cáscara de Punica granatum L con posible utilidad para el tratamiento de la diabetes mellitus. Tesis para obtener el grado de Doctora en Biología Experimental, pp. 153

Number 3 Issue 1 February 2013

full text provided by www.ibcj.org.mx

International Biotechnology Color Journal

A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY and Covering Its Many Hues, Tints, Tones & Shades

Number 3 Issue 1 February 2013

full text provided by www.ibcj.org.mx

International Biotechnology Color Journal

A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY and Covering Its Many Hues, Tints, Tones & Shades

MISSION

The **International Biotechnology Color Journal** (*IBCJ*) is the official trimonthly scientific Journal of the International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society A.C., a nonprofit corporation.

IBCJ is devoted to facilitating the advancement of our understanding of Biotechnology in its broader definition: The application of science and technology to living organisms, as well as parts and models thereof, to alter living and non-living materials for the production of knowledge, goods and services.

IBCJ is committed to publishing original contributions of research in all areas related to the theory and practice of biotechnology in its broadest context (organized by color), including research articles and notes, critical reviews, essays, book reviews, letters, correspondence, and news features or views.

IBCJ intends to provide an excellent resource for the publication of peer-reviewed research papers with proven or likely implications for the past, current, and future practice of biotechnology.

AUTHOR GUIDELINES

IBCJ will include current trends in scientific publication for the electronic format and international requirements for indexing. All manuscript should have a corresponding author. The corresponding authors assumes full responsibility for the published data, on behave of all authors.

Authors must transfer the copyright of their article for International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society A.C.

For review, manuscripts should be submitted to the *IBCJ* Chief editor, by e-mail, at:

"Dr. José Juan Zúñiga Aguilar" *<zuniga@cicy.mx>*

Manuscripts submitted to the *IBCJ* will be assigned to one the scientific *IBCJ* Editor, according to the section suggested by the corresponding author. The *IBCJ* Scientific editor will determine if the manuscript is within the journal scope, has high scientific quality, is presented in a manner suitable for publication in a scientific peer-reviewed journal, the content has not been published elsewhere, and if it has not been previously rejected by this journal. If the submission is considered to meet all of the above criteria, it will be forwarded to additional to referees with enough expertise in the field, for further review. Referee's names will not be disclosed, but their comments will be forwarded to the authors.

Area colors and their scope

RED	Medical Biotechnology:	*human health & disease, novel medical diagnostics and tissue engineering.*
YELLOW	Nutritional Biotechnology:	*Food, nutrition science and neutraceuticals.*
BLUE	Marine and Fresh Water Biotechnology:	*Aquaculture and fisheries; coasts and sea; fish and aquatic animals: health, nutrition, reproduction, cloning and genetic modifications, pests and disease control*
DARK GREEN	Agricultural Biotechnology:	*Biotechnology for the cultivation, processing, and storage of plants. Plant tissue culture and micropropagation. Biofertilizers, agrobiochemicals, and plant pests and disease control. Biotechnology in plant ecology and biodiversity;*
SOFT GREEN	Animal Biotechnology:	Biotechnology for the production, processing, and storage of livestock. Pets and farm-animals disease, health, nutrition, reproduction, cloning, and genetic modification.
LIME GREEN	Biotechnology of Renewable Resources:	*Bioremediation & environmental Biotechnology. Biofuels and renewable bioenergy sources, and bioenergy sources. Biotechnology and sustainable development, competitive production, biomaterials.*
BROWN	Desert Biotechnology:	*Arid zone & desert Biotechnology; space and Geomicrobiology.*
DARK	Bioterrorism:	*Human and animal pathogen manipulation, bioterrorism, biowarfare, biocrimes, anticrop warfare*
PURPLE	Patents, IPR:	*Strategy for intellectual property protection, patents, publications, inventions*
WHITE	Biotechnology of GMO's:	Industrial Biotechnology of Genetically Modified Organism's. *Gene-based industrial applications*
GOLD	Bioinformatics:	*Computer applications to the study of biological systems. Micro and nanobiotechnology, mixed biological- electronic/electromechanical systems and biomachines (MEMS and NEMS, MST and NST).*
GREY	Fermentations and Classical Biotechnology:	*Industrial biotechnology: classical fermentation & bioprocess/bioengineering technology; engineering and technological equipment for bioproduction; output of science-intensive bioproducts*
IRIS	Multidisciplinary area:	*Biochemistry, molecular biology & biotechnology, Omics applications*
PLATINUM	Synthetic Biology	*Design and obtention of new biological components, devices, and systems. Applications of re-designed natural biological systems.*
SILVER	Biobusiness, Bioentrepreneuship & Marketing:	*Development economics, Biobusiness and marketing. Strategy for innovative economical development. Improvement of the system of the S&T and innovation activities management*
TRANSPARENT	Biotechnology, Bioethics & Society:	*Assesment of the public support to the scientific activity. Biotechnological potential and human resources*
INDIGO	Integrating science, education and manufacturing:	*Education & early childhood stimulation in the culture of health, nutrition, sport, art, science, biotechnology & society as information and telecommunication technologies TIC´S*

Authors should suggest the section in which their work is best placed form, and should indicate the type of work submitted form:

- CONTRIBUTION ARTICLES - NOTE - ESSAY - BOOK REVIEW - LETTERS TO THE EDITOR - CORRESPONDENCE - NEWS AND VIEWS.

About IFFBRAESITCOHNSASTAS AC Non Profit.

The International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society A.C. was founded in 2009 as a non-profit association to promote the development of professionals of biotechnology in research, industry, services, and education, and for the diffusion of biotechnological knowledge. Organizes the Biotechnology summit and Biotechnology color meeting for education that includes activities of current interest for the professionals and students working or interested in the areas of biotechnology. It also promotes the area of research, education, industry, and technological development. The International Biotechnology Color Journal (IBCJ) is the official trimonthly Journal of the International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society since 2011 and is devoted to the advancement of our understanding of Biotechnology (*The application of science and technology to living organisms, as well as parts and models thereof, to alter living and non-living materials for the production of knowledge, goods and services*).

Important note

These instructions for authors will appear periodically at the end of an issue, and will be available form the Journal's website. Please consult the latest instructions published, as they may change to meet new journal's requirements.

CALL FOR PAPERS

Make

International Biotechnology Color Journal

the forum for your research

Peer reviewed

Recognized editorial board

Electronic

No charges

Broad Scope

Great design

Small staff

Just starting

Small size

MEANS

Original contributions of high scientific quality

Your contribution, if accepted, will be trusted by your colleagues

It can be accessed in every country

Everybody can read your work, at no cost for you

An audience with diverse scientific backgrounds

Its attractive design will enhance your work's visibility

No bureaucratic intermediacy, you can contact us directly

We are sensible, sensitive to your needs, and willing to help

Your CONTRIBUTION will not get lost inside an overwhelming number of papers

How can this be true?

Our staff and editorial board are generous academics, who understand the value of accessible, but trustworthy scientific communications

CONTACT US: sotres@unam.mx, zuniga@cicy.mx

WE HOPE TO HEAR FROM YOU SOON !

International Biotechnology Color Journal
A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY
and Covering Its Many Hues, Tints, Tones & Shades

DISCLAIMER

The content of the scientific contributions has been entirely generated by the contributing authors of each article.

The data behind each contribution is assumed to be truthful, and the IBCJ staff has no means to verify their validity.

The printed content of this Journal is protected as indicated below. However, the authorship of the data supporting the contents is still the intellectual property of each corresponding author and coworkers. IBJC assumes no legal responsibility for experiments, data analysis, or ideas included on any of the contributions.

This journal is only produced in electronic form.

The production and distribution of this Journal is non-lucrative. No fees are charged to contributing authors or subscribers.

The following software was employed in its production:

Open Office™ The Apache Software Foundation
(http://www.openoffice.org/).
Scribus© 2001-2010 Franz Schmid and the members of the Scribus Team
(http://www.scribus.net).
The GIMP© 2001-2011 The GIMP Team
(http://www.gimp.org/).

The Editorial board thanks to the individuals involved in the generation of the software.

Number 3 Issue 1 February 2013

full text provided by www.ibcj.org.mx

International Biotechnology Color Journal

A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY and Covering Its Many Hues, Tints, Tones & Shades

Number 3 Issue 1 February 2013

full text provided by www.ibcj.org.mx

International Biotechnology Color Journal

A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY and Covering Its Many Hues, Tints, Tones & Shades

The International Biotechnology Color Journal appears quarterly, starting in October 2011. It is a non-profitable Electronic Publication. Currently, it does not change publication or access fees. It publishes Original Scientific Information, previously unpublished, provided by contributing authors, out of their own will. Produced and Hosted by Centro de Investigación Científica de Yucatán, A.C., Calle 43 No. 130, Colonia Chuburná de Hidalgo. Mérida Yuc., Mexico. Tel: (+52) 999 942 8330. Fax: (+52) 999 942 3900. RFC. CIC791116770. The staff does not earn a salary for the activities associated to this Journal's production. **Int. biotechnol. color j., ISSN 2226-0404.**