UADY

POSGRADO INSTITUCIONAL EN CIENCIAS QUÍMICAS Y BIOQUÍMICAS

# EFECTO HIPOGLUCÉMICO E HIPOTENSOR DE EXTRACTOS DE HOJAS DE CHAYA (*Cnidoscolus aconitifolius*) EN ESTUDIOS *in vitro* E *in vivo.*

## TESIS

PRESENTADA POR

María Lilibeth Manzanilla Valdez

EN OPCIÓN AL GRADO DE

MAESTRA EN CIENCIAS QUÍMICAS Y BIOQUÍMICAS

MÉRIDA, YUCATÁN, MÉXICO

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## RESUMEN

Las enfermedades crónicas no transmisibles (ECNT) son la principal causa de mortalidad en México y el mundo. La hiperglucemia, el exceso de peso y la hipertensión son factores de riesgo en la ECNT. La fitoterapia presenta una alternativa al tratamiento de las ECNT. La chaya (*C. aconitifolius*) es un arbusto cultivado en la región Maya y, se ha empleado en la medicina tradicional desde la época prehispánica; sus hojas han reportado efectos biológicos como antioxidantes, hipolipémicos y antibacterianos. El objetivo de este trabajo fue, evaluar el efecto hipoglucémico e hipotensor de los extractos de hojas de chaya (*C. aconitifolius*) en estudios *in vitro* e *in vivo*. Así, se elaboraron seis extractos; agua, etanol, acetona, acetato de etilo, éter dietílico y hexano (1:10 p/v), por maceración por 48 h. Todos los extractos se evaluaron biológicamente. Se evaluaron tres ensayos *in vitro*; inhibición de la enzima convertidora de angiotensina (ECA), inhibición de la enzima $\alpha$-glucosidasa y $\alpha$-amilasa. En el estudio *in vivo*, se utilizaron ratas macho Wistar con obesidad, hiperglucemia e hipertensión y, se determinó la curva de tolerancia a la glucosa (CTG), el efecto hipoglucémico y el efecto hipotensor. *In vitro,* el extracto de acetona tuvo el menor valor de $CI_{50}$ en la ECA con 12.61 µg/mL y, el extracto de acetato de etilo tuvo el menor $CI_{50}$ en la $\alpha$-amilasa y $\alpha$-glucosidasa con 22.97 y 3.20 µg/mL. El extracto acuoso tuvo el mayor efecto hipotensor al disminuir la presión arterial sistólica y diastólica en 17.3 y 23.4%, respectivamente. En la CTG a 0.5 mg/kg el extracto hexánico fue el único que disminuyó la glucosa (7.6 %). En el efecto hipoglucémico a 5.0 mg/kg, presentó una disminución de la glucosa del 22.9 %. En conclusión, los resultados exhiben el valor biofuncional de los extractos de chaya, en específico del extracto acuoso y hexánico por su mayor efecto hipoglucémico e hipotensor.

## ABSTRACT

Noncommunicable diseases (NCDs) are de main cause of mortality in Mexico and the world. Hyperglycemia, excess weight and hypertension are risk factors in developing NCDs. Phytotherapy presents an alternative to treatment of NCDs. Chaya (*C. aconitifolius*) is a shrub cultivated in the Mayan region and has been used in traditional medicine since pre-hispanic times, it´s leaves have reported biological effects as antioxidants, hypolipids and antibacterial. The aim of this work was to evaluate the hypoglycemic and hypotensive effect of extracts of Chaya leaves (*C. aconitifolius*) with *in vitro* and *in vivo* assays. Thus, six extracts were prepared; water, ethanol, acetone, ethyl acetate, diethyl ether and hexane (1:10 p/v), by maceration for 48 h. All extracts were evaluated biologically, three *in vitro* tests were performed; inhibition of angiotensin converting enzyme (ACE), inhibition of the $\alpha$-glucosidase and $\alpha$-amylase enzyme. In the *in vivo* study, male Wistar rats with obesity, hyperglycemia and hypertension were used. The glucose tolerance curve (GTC), the hypoglycemic effect and the hypotensive effect were determined. *In vitro;* the acetone extract had the lowest $IC_{50}$ of ACE with 12.61 µg/mL, and the ethyl acetate extract had the lowest $IC_{50}$ $\alpha$-amylase and $\alpha$-glucosidase with 22.97 and 3.20 µg/mL. The aqueous extract of chaya had the highest hypotensive effect by decreasing systolic and diastolic blood pressure by 17.3 and 23.4 %, respectively. In the GTC at 0.5 mg/kg the hexane extract was the only one that decreased blood glucose levels by 7.6 %. In the hyglycemic effect at 5.0 mg/kg, there was a 22.9 % decreased in blood glucose. In conclusion, the results shown the biofunctional value of chaya extracts, specifically the aqueous and hexane extracts, due to their greater hypoglycemic and hypotensive effect.

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## INTRODUCCIÓN

Uno de los mayores retos en la actualidad son las ECNT; éstas son la principal causa de mortalidad en México y en el mundo toda vez que, el 71% de muertes al año son a causa de estos padecimientos. Las ECNT son enfermedades crónicas de larga duración y son la combinación de diferentes factores tales como: exceso de peso, sedentarismo, mala alimentación y genética. La Organización Mundial de la Salud (OMS) ha establecido los principales tipos de ECNT: enfermedades cardiovasculares, el cáncer, las enfermedades respiratorias crónicas y la diabetes mellitus (DM). Existen diversos factores de riesgo que favorecen al desarrollo de las ECNT, los modificables y los metabólicos. Los factores modificables son: el sedentarismo, la alimentación y el consumo de alcohol y tabaco. Por otro lado, se encuentran los factores de riesgo metabólicos tales como: el exceso de peso, la hipertensión arterial, la hiperglucemia y la hiperlipidemia. La mayoría de estas enfermedades podrían evitarse mediante estrategias eficaces y de bajo costo.

En México, el principal factor de riesgo de las ECNT es la hipertensión arterial sistémica (HAS) seguido de la DM. Las encuestas de nutrición realizadas a través de los años (1986-2016) señalan el aumento en la prevalencia de la hiperglucemia, la DM y de la HAS (ENSANUT MC 2016; INSP, 2018). La HAS se caracteriza por el aumento sostenido en la presión arterial (PA) sistólica >130-139 mmHg y la PA diastólica >80-89 mmHg (American Heart Association, 2017). Este aumento causa vasoconstricción en el endotelio vascular, reduciendo la luz de las arterias y forzando al corazón a aumentar la PA. La HAS puede causar infartos al miocardio, infartos cerebrales, enfermedad renal crónica y pérdida de la visión (Campos-Nonato *et al*., 2018; Taler, 2018). Por su parte, la DM tipo II se caracteriza por el aumento en los niveles de glucosa en ayuno >120 – 140 mg/dL y por la disminución en la cantidad de insulina secretada por las células β-pancreáticas. La resistencia a la insulina causa DM, además es un trastorno sistémico que afecta a diferentes órganos (músculo, adipocitos, hígado) y a las vías de señalización reguladas por dicha hormona. Además, hay una reducción de la acción de la insulina en los tejidos dependientes de ésta, a pesar del aumento en la secreción de dicha hormona en el organismo (Heymsfield y Wadden, 2017; Petersen *et al*., 2018).

El tratamiento nutricional y farmacológico es indispensable en las enfermedades crónicas (Peralta, 2016). Es importante mencionar que, éste último conlleva a efectos secundarios nocivos para la salud, ya que causa diarrea, nauseas y pérdida del apetito. Otro factor por considerar es, el costo de los medicamentos, ya que actualmente no hay un solo medicamento que tenga efecto hipotensor e hipoglucémico al mismo tiempo.

En México, el uso de plantas como alternativa natural ha sido usada desde la época prehispánica. Se han estudiado el efecto de diferentes plantas tales como: *Silybum marianum* (cardo mariano), *Rosmarinus officinalis (*romero), *Synara scolymus* (alcachofa) y *Taraxacum officinale* (diente de león), ofreciendo protección contra la hepatitis, ictericia, hígado graso, cirrosis y con efectos antioxidantes (Andrade-Cetto y Heinrich, 2005). Estos

efectos se deben a los metabolitos secundarios (MS) presentes en las plantas. Los MS son compuestos químicos sintetizados por las plantas y tienen efectos biológicos positivos en el organismo (Mata *et al*., 2013). Debido a esto, la fitoterapia representa una alternativa de tratamiento para las ECNT. En el sureste de México se encuentra la chaya (*C. aconitifolius*); un arbusto cultivado en toda la región Maya, que pertenece a la familia *Euphorbiaceae* y al género *Cnidoscolus*; ésta puede crecer hasta seis metros de altura, con hojas de 32 cm de largo y 30 cm de ancho; existen cuatro variedades de *C. aconitifolius*: estrella, picuda, redonda y mansa, siendo esta última de uso comestible y medicinal en Yucatán (Ross-Ibarra et al., 2003). Diversos autores han reportado los efectos biológicos en las hojas de *C. aconitifolius,* tales como antioxidantes, hipolipemiantes y antibacteriales (Oyagbemi *et al*., 2013; García-Rodríguez et al., 2014; Ramos-Gómez *et al*., 2016), siendo una posible alternativa para el tratamiento y prevención de las ECNT. Por lo anterior, el objetivo de este trabajo fue, evaluar el efecto hipoglucémico e hipotensor de los extractos de hojas de chaya (*C. aconitifolius*) en estudios *in vitro* e *in vivo*.

## HIPÓTESIS

Los extractos obtenidos de las hojas de chaya (*Cnidoscolus aconitifolius*) tienen efecto hipoglucémico e hipotensor en estudios *in vitro* e *in vivo*.

## JUSTIFICACIÓN

Las ECNT, son enfermedades de larga duración y son el resultado de la combinación de factores genéticos, fisiológicos, ambientales y conductuales. Las ECNT matan a 41 millones de personas al año, siete de cada diez personas en el mundo padecen ECNT y, se estima que para 2030 sean 55 millones de personas que mueran al año por estos padecimientos. Los factores de riesgo que aumentan el desarrollo de ECNT son hipertensión, sobrepeso, obesidad, hiperglucemia e hiperlipidemia. Con la finalidad de disminuir el impacto de las ECNT, la OMS ha propuesto emplear un enfoque integral y preventivo (OMS 2018; INSP 2018).

En México, la falta de un sistema preventivo ha ocasionado el aumento de ECNT, tales como la DM tipo II y las enfermedades cardiovasculares secundarias a la HAS. La DM y la HAS ocupan el primer y segundo lugar de mortalidad en México (AVISA 2015; OMNET 2019). El sector salud es el encargado de tomar acciones preventivas y brindar tratamiento farmacológico a los pacientes. Las desventajas del tratamiento farmacológico son amplias, una de ellas son los efectos secundarios que se pueden presentar, tales como náuseas, mareos, vómitos, fatiga, pérdida del apetito y diarrea, los cuales causan incomodidad en los pacientes (Luyckx, 2012). Debido a esto, se han buscado alternativas naturales que no tengan efectos secundarios hacia los pacientes. Una posible opción son los MS, que están presentes en diversas plantas (Hanbing Li *et al*., 2017).

La chaya (*C. aconitifolius*) es una planta comestible ampliamente consumida en la península de Yucatán, de bajo costo y gran accesibilidad y, ha sido utilizada desde la época prehispánica por sus propiedades medicinales (Ross-Ibarra *et al*., 2003; Ramos-Gomez *et al*., 2016). Los extractos derivados de esta planta han demostrado tener actividades con potencial biológico positivo, sugiriendo su posible uso como coadyuvante en el tratamiento de la hiperglucemia, resistencia a la insulina, DM y HAS (Koty y Konuro 2004; Oyagbemi *et al*., 2013; Ramos-Gomez *et al*., 2016). El presente estudio evaluó el efecto hipoglucémico e hipotensor de los extractos de hojas de chaya (*C. aconitifolius*) como alternativa al planteamiento de nuevos tratamientos nutricionales o como posible coadyuvante en el tratamiento farmacológico de la hiperglucemia e hipertensión arterial.

## OBJETIVOS

**Objetivo general:**

Evaluar el efecto hipoglucémico e hipotensor de los extractos de hojas de chaya (*Cnidoscolus aconitifolius*) en estudios *in vitro* e *in vivo.*

**Objetivos específicos:**

1. Determinar la actividad inhibitoria de la enzima convertidora de angiotensina (ECA) *in vitro* de los extractos de *C. aconitifolius*.
2. Determinar la actividad inhibitoria de la enzima α-glucosidasa y α-amilasa *in vitro* de los extractos de *C. aconitifolius*.
3. Evaluar el efecto hipotensor *in vivo* de los extractos de *C. aconitifolius* en un modelo murino con hiperglucemia y HAS.
4. Determinar el efecto antihiperglucémico de los extractos de *C. aconitifolius* mediante la curva de tolerancia a la glucosa inducida con almidón en un modelo murino con hiperglucemia y HAS.
5. Determinar el efecto hipoglucémico de los extractos de *C. aconitifolius* en un modelo murino con hiperglucemia y HAS.

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**CAPÍTULO DE LIBRO**

# "Current Status of Renal and Hepatic diseases: *Cnidoscolus aconitifolius as an alternative for therapy and prevention"*

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## ABSTRACT

The lack of prevention of Noncommunicable diseases (NCDs) has caused an increase in the mortality rate including conditions such as chronic kidney disease (CKD) and liver disease (LD). The high complexity of CKD and LD results in alterations in the metabolism of carbohydrates, proteins and lipids. One of the changes observed in CKD and LD is the decrease in albumin, elevation of $PO_4^{-3}$, $K^+$, creatinine, urea and transaminase enzymes. The pharmacological treatment is expensive. Nowdays, phytotheraphy is an option to treat NCDs. Aqueous, ethanolic, methanolic and ethyl acetate extracts of *Cnidoscolus aconitifolius* have shown nephroprotective and hepatoprotective potential and can be an alternative to prevent and treat CKD and LD. Chaya (*C. aconitifolius)* is a shrub that is consumed in Mexico and in the world, has a low cost, it´s very accessible, and can growth in extreme weather. The aim of this chapter is to show the potential biological effects of *C. aconitifolius* extracts, and the association of the phytochemicals in the extract. It is known that different solvents result in the uptake of different phytochemicals. These have shown various effects such as hypoglycemic, hypotensive, hypolipidemic and antioxidant, being a natural alternative to the treatment of NCDs.

**Keywords:** Chaya, *Cnidoscolus aconitifolius*, hepatoprotective, nephroprotective, NCD, insulin resistance.

**1. NCDs as a global problem and its consequences**

NCDs are long-term diseases with a slow progression, among which there are five main types: cardiovascular diseases, cancer, chronic respiratory diseases, diabetes mellitus (DM) and CKD. There are four external risk factors, that can be associated to the development of these diseases: tobacco consumption, malnutrition, sedentarism and alcohol intake. Metabolism problems such as hypertension, overweight and obesity, hyperglycemia, dyslipidemias and insulin resistance are risk factors that increase the predisposition of NCDs (WHO, 2013, 2017).

The National Health Report of Mexicans (AVISA) of 2015 states that the first cause of death in the country is DM, followed by ischemic heart disease and third CKD. In recent decades, the changes in lifestyle such as stop walking and spend more time in the car, low intake of fruits and vegetables, the increase in the consumption of foods with high energy content, and obesity are determining factors in the development of NCDs (Clark et al., 2013).

The health and nutrition research that have been conducted from 1988 to 2016 have shown the epidemiological panorama in Mexico and its evolution. First, the problems of acute and chronic undernutrition in children under five years reported by the National Nutrition Survey (NNS) of 1988, and then in 2016 the ENSANUT shows the problems that comes from obesity such as insulin resistance, diabetes mellitus (DM) and hypertension. The epidemiological transition in the country is evident, in the seventies and eighties the problem of mortality was related to undernutrition and infectious diseases such as pneumonia, intestinal infections and pulmonary tuberculosis (NNS, 1988) and in the studies carried out since 2013 by the National Institute of Public Health indicates the increase in NCDs as a consequence of obesity.

Every year, the NCDs kills 40 million people in the world, of which 37.5% are people between the ages of 30 to 69. Cardiovascular diseases constitute the majority of deaths with 17.7 million, then cancer with 8.8 million, respiratory diseases with 3.9 million and DM with 1.6 million (WHO, 2017). In most of these diseases the common factor is obesity and insulin resistance. In 2017 the “Obesity Update” reported that one in two adults suffers from overweight and obesity, and in children one in six suffers from excessive weight in the world. The countries with the highest prevalence of obesity are: United States of America with 38.2%, Mexico 32.4%, New Zealand 30.7% and Hungary 30%. By 2030, these numbers are expected to increase, with a projection than 45% in the United States of and more than 35% in Mexico.

In the 2016 ENSANUT reported in Mexico that, 33.2% of school-age children are overweight and obese, and that 72.5% of adults over 20 years of age are overweight. The DM presented a prevalence of 9.4%. While, 25.5% of the population suffers from hypertension; it was observed that the prevalence of obesity, DM and hypertension was higher in women than in men.

DM is the leading cause of death in Mexico, an estimated 80 thousand deaths per year (Mexico National Institute of Health, 2017). DM has multiple complications such as: diabetic nephropathy, blindness, myocardial infarction, ischemic brain infarction (IBI) and lower limb amputations that contributes to increased mortality (WHO, 2017). DM type 2 and hypertension are the main causes of CKD (Espinosa-Cuevas, 2016). In 2008, the prevalence of CKD in Mexico City was 22% and in Jalisco 33% (Keep, 2008). On the other hand, liver diseases accounted for the second highest mortality in men aged 30 to 44 years and in women aged 45 to 59 years are the third cause (INEGI, 2015).

Due to the approach to treatment and the lack of prevention that exists in the country, the sustainability of the health system is decreasing. In Mexico, the annual expenditure is 155 billion pesos, this is divided between the levels of health care, and it is estimated that by 2050 it will be 344 billion pesos (IMSS, 2016). For its part, the institute of safety and social services for employees of the state (ISSSTE) reports that the cost of CKD and replacement therapy in 2017 fluctuated from 25 to 35 thousand pesos per beneficiary. On the other hand, renal transplantation and hemodialysis (HD) plus the medicines can cost 2 million pesos per patient, being the Mexican Social Security Institute (IMSS) and the social insurance (SS) the dependencies with more people in the organs waiting list (National Transplant Center, 2016). In Mexico, more than 15 billion pesos are spent annually to attend to NCD, specifically cardiovascular diseases, DM, cervical cancer, breast cancer and CKD. In order to reduce the costs of these diseases before the health system collapses, a preventive approach is needed (IMSS, 2016).

As mentioned before CKD originates from glucose imbalance, insulin resistance, DM and hypertension. Meanwhile, the LD can occur form different mechanisms. In most cases, CKD and LD progress quietly and are not diagnosed until they reach a terminal chronic stage, in which the patient already needs replacement therapy or transplantation (Ávila-Saldívar et al, 2013; Sarmiento-Quintero et al, 2016).

The CKD causes irreversible damage to the structure of the kidney, chronicity causes imbalances in the mineral and bone metabolism, as well as total energy expenditure (TEE). The lack of an adequate dietary treatment affects the organism, causing a protein energy wasting (PEW), which aggravates the symptoms of CKD and as a result of this, the pharmacological treatment increases. Thus, CKD is a complex disease that begins in the kidney, its complications spread throughout the body. Therefore, it is necessary to know the functioning of the kidney to understand its abnormalities.

One of the main complications of LD in the world is non-alcoholic fatty liver disease (NAFLD) and it is associated with obesity, DM and dyslipidemias. Excessive weight, insulin resistance, metabolic syndrome and a sedentary lifestyle are risk factors that can increase the NAFLD (Bernal-Reyes, 2015). NAFLD is defined as the infiltration of fat of more than 5% to the hepatocyte, among the complications includes the development of cirrhosis, fibrosis or hepatocarcinomatous. The treatment for this condition is focused on a healthy diet and physical activation (Sarmiento-Quintero et al, 2016).

## 2. Physiology and renal anatomy

One of the most important organs in the body are the kidneys, these are located near the middle part of the back, on both sides of the spine, measuring approximately 12 cm in length and 6 cm in width. Their main function is to eliminate waste from the body through urine. There are different functions of the kidneys, there are described above (Modified by Guyton-Hall, 2016; Mataix-Verdú, 2007).

1) Excretion of metabolic products.
2) Regulation of water and electrolyte balances.
3) Regulation of the osmolarity of body fluid and electrolyte concentrations.
4) Regulation of blood pressure.
5) Regulation of acid-base balance.
6) Endocrine function:
   a) Synthesis of erythropoietin (EPO): stimulates the bone marrow to produce red blood cells.
   b) Synthesis of 1,25 dihydroxycholecalciferol (vitamin D): This substance is key in the metabolism of calcium.
   c) Renin synthesis: powerful vasoconstrictor with which regulates the blood pressure system.
   d) The degradation of peptide hormones such as parathyroid hormone (PTH), calcitonin, insulin, glucagon and gastrin.

### 2.1 Excretion and kidney regulation

In order to eliminate toxins, it is necessary to excrete metabolites, this process is done by nephrons. The kidney has around one million nephrons, inside them there is the glomerulus (a blood vessel and capillaries) that connects with the tubules and then collects the urine and transports it to the bladder (NIDDK, 2009). It is important to mention that although there are many nephrons, they cannot be regenerate. Therefore, once a nephron suffers a damage (due to progress of some pathology) this nephron will be lost, due to the above it is important to take care of the kidneys, due to the danger of developing chronic diseases. In Figure 1, the structure of the kidney is presented, it can be seen that through the renal vein it reaches the blood with metabolism products such as urea, creatinine, uric acid, products of the metabolism of hemoglobin and the metabolites of various hormones. These products are eliminated in the urine through the ureter, an excess of urea and uric acid has negative consequences in the organism, it can cause systemic toxicity. Once filtered and without toxins, blood returns to the organism through the renal artery (Guyton-Hall, 2016).

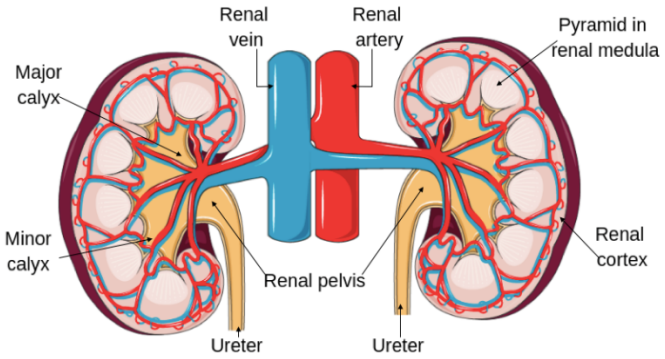


**Figure 1.** Structure of the kidney

Another important function of the kidneys is the regulation of the hydro-electrolytic balance, this is done by the excretion of water and electrolytes such as ions like chlorine ($Cl^-$), potassium ($K^+$), calcium ($Ca^{+2}$), hydrogen ($H^{+)}$, magnesium ($Mg^{+2}$) and phosphorus ($PO_4^{-3}$). The purpose of this system in the kidney is to maintain homeostasis in the body and also to regulate the intake of water in the body. The regulation of water intake is related to plasma osmolality; the hypothalamus detects the changes of osmolality, which activates the sensation of thirst and the secretion of the antidiuretic hormone (ADH). In CKD, the reabsorption of sodium ($Na^+$) increases, as a consequence the extracellular volume changes and urinary excretion diminishes, which results in edema (Alcázar-Arroyo, 2008). Edema is very common in CKD; this is why it is necessary to have an accurate control of fluid intake. The WHO determined that the recommended daily intake (RDI) of $Na^+$ for the adult population is 1600 mg per day. For its part, the study conducted in 2009 by the Mexican Institute of Medical Science and Nutrition Salvador Zubirán (IMMNSZ), estimated that the daily consumption of salt in Mexico is 9 g in men and 7 g in women, noticing that Mexicans have a high consumption of sodium, and how dangerous a diet with this characteristics is. Diets with a high $Na^+$ content stimulate electrolyte imbalance in CKD.

Renin is a proteolytic enzyme that is synthesized in the kidney and participates in the renin-angiotensin-aldosterone cycle. This synthesis is responsible for changes in volume and pressure. Renin acts on the angiotensinogen that is released from the liver and when is coupled, angiotensin I is obtained. Subsequently, the pulmonary endothelium releases the angiotensin-converting enzyme, which synthesizes angiotensin II (Figure 2). Angiotensin II is responsible for vasoconstriction, through the mechanism of intracellular sodium retention, promoting the increase of serum osmolarity. Angiotensin II also stimulates the synthesis of aldosterone and stimulates proximal sodium reabsorption. This enzyme has two types of receptors I and II, the increase in angiotensin II concentrations has as a consequence, greater coupling to its receptors, this translates into greater systemic vasoconstriction and increase in systemic arterial pressure, causing hypertension. Most of the pharmacological treatments block the type I receptors, these

decrease the hypertension, by releasing nitric oxide and activating the tumor necrosis factor beta, which is an important pro-inflammatory factor (Méndez-Durán, 2011).

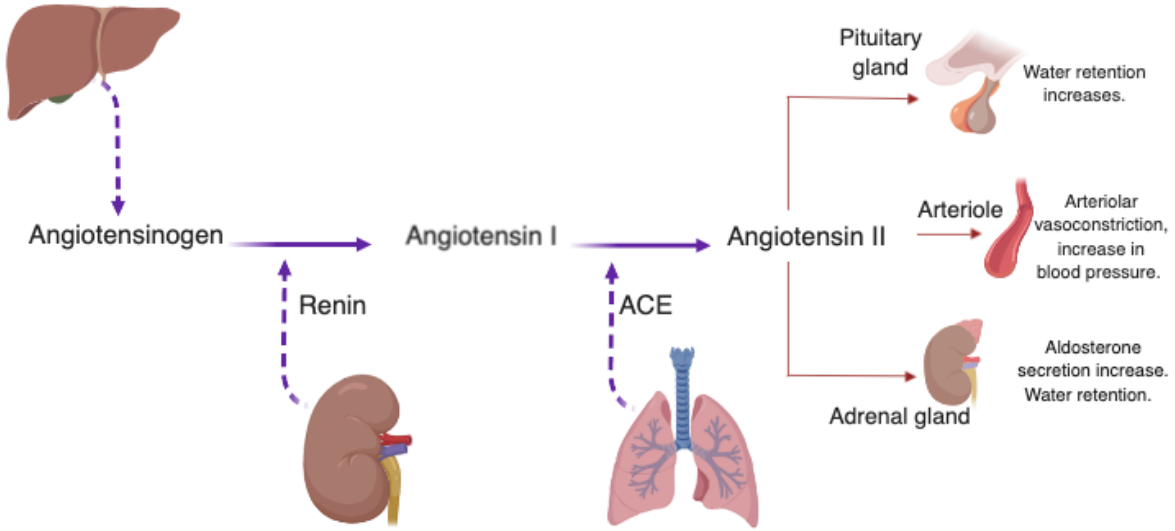


**Figure 2.** Renin-angiotensin-aldosterone cycle.

The kidneys and the lungs are the main organs in charge of acid-base regulation. The sulfuric acid and the phosphoric acid that are generated in the metabolism of the proteins, are eliminated from the organism by the kidney. In acidosis, the anion gap is usually increased by the accumulation of phosphate and sulfate anions. In the patient with CKD, moderate metabolic acidosis is common and stimulates bone demineralization, extracting $Ca^{+2}$ and $PO_4^{-3}$ from bone, these minerals act as buffers for excess acid, to maintain chemical balance in the body. The consequences to other organs are secondary pulmonary hyperventilation and cardiac muscle atrophy. It has also been related to metabolic acidosis with decreased serum albumin levels and insulin resistance (Alcázar-Arroyo, 2008).

### 2.2 Endocrine function of the kidney

One of the main endocrine functions in the kidney is the synthesis of erythropoietin (EPO), the aim of EPO is the maturation of erythrocytes. Deficiency in its synthesis results in anemia. Calcitriol is also an important part of the endocrine function, the decrease in the calcitriol parameters result in reabsorption of $Ca^{+2}$, these can be manifested in the body in osteoporosis. Finally, in situations of extreme fasting the kidney has the ability to synthesize glucose to preserve life. These functions are discussed below;

a) Synthesis of EPO. EPO is a glycoprotein hormone (glycoprotein), which stimulates the production of erythrocytes by hematopoietic stem cells in the bone marrow. The erythrocytes are responsible for transporting oxygen to the blood, its production and regulation occur mainly in the kidney. The synthesis of EPO sends the signal to the bone marrow and to the stem cells to promote the correct maturation of the erythrocytes (Figure 3). Anemia is persistent in 90% of patients with CKD and is due to the low production of endogenous EPO (Cabrera-García et al, 2009). The

decrease of oxygen in the tissues, creates chronic fatigue and intolerance to the effort.

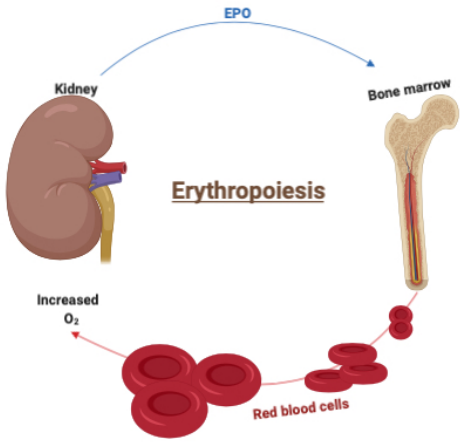


**Figure 3.** Synthesis of erythropoietin. EPO promotes production of mature red blood cells into the bone marrow.

b) Synthesis of 1,25-dihydroxycholecalciferol (calcitriol). The kidney regulates the reabsorption of $Ca^{+2}$ and $PO_4^{-3}$, as well as the synthesis of 1,25-dihydroxycholecalciferol. The renal regulation of vitamin D metabolism is due to the calcitriol that is bound to the transport protein, passes through the glomerulus and is captured by the proximal tubule by endocytosis. Upon reaching the mitochondria it binds to 1-α-hydroxylase. Parathyroid hormone (PTH) and growth factor 23 (FGF-23) directly influence this mechanism, the first as a mediator of $Ca^{+2}$ and the second as a mediator of $PO_4^{-3}$ (Figure 4). The active form of vitamin D is very important for the deposit of $Ca^{+2}$ in the bone and for its reabsorption at the intestinal level.

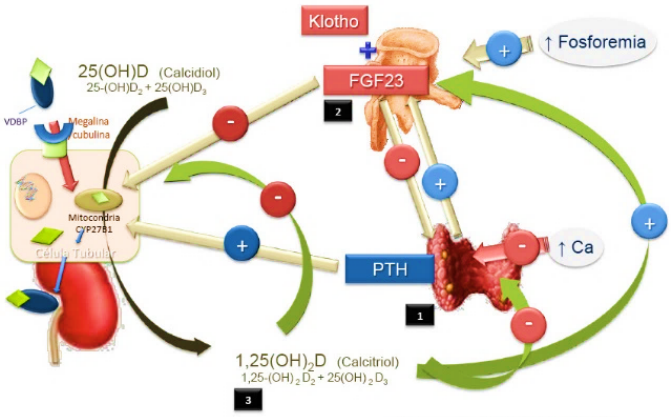


**Figure 4.** Synthesis of calcitriol (Taken from González-Casaus, 2012)

c) Synthesis of glucose. In situations of prolonged fasting, the kidney can synthesize glucose from amino acids, this process is called gluconeogenesis. In CKD this function is interrupted causing anomalies in the volume of body fluid. Gluconeogenesis is a route that consumes energy, 6 ATP are consumed for each

molecule of glucose. Proteins and lipids such as glycerol, propionyl CoA and pyruvate from the carbohydrate pathway participate in this route. The final reaction is the dephosphorylation is glucose-6-phosphate, catalyzed by glucose-6-phosphatase in a hydrolysis reaction (Figure 5) (Alarcón-Sotelo et al., 2015).

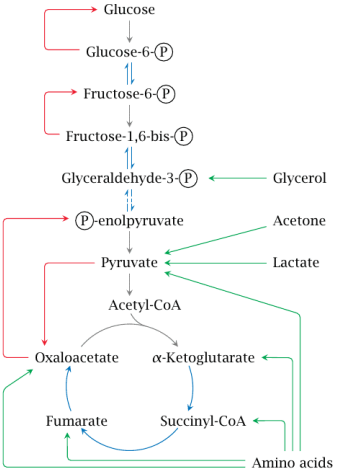


**Figure 5.** Gluconeogenesis

### 3. The renal pathophysiology a glance of CKD

Renal pathology is very extensive and complex, since it involves different organs and complications. Different diseases can cause renal complications, the lack of control in blood glucose (insulin resistance and DM) is the main cause of CKD, followed by complications of hypertension (Calvo-Vázquez et al., 2015). Insulin resistance produces vascular damage inducing proliferation in the smooth muscle and affects the sensitization of angiotensin II (Ardiles and Navarro, 2015). On the other hand, patients with DM have accumulation in the extracellular matrix, affecting the basal membrane of the glomerulus, the mesangio in the tubular basal membrane and in the interstitium. Changes in glomerular filtration rate, (GFR) such as sustained hyperfiltration and albuminuria in DM is the first sign of kidney disease (Ávila-Saldívar et al., 2013). In Figure 6, it can observe the two types of damage that are caused at the endothelial and mesangial levels in the nephron. The endothelial damage in the epithelium and the increase in the mesangial matrix results in damage to the glomerulus, developing glomerulosclerosis. On the other hand, the mesangial damage increases the permeability in the glomerulus, resulting in albuminuria. Albumin is an indicator of nutritional status at a cellular level. Low serum albumin levels indicate persistent cellular malnutrition, albumin is an important parameter since it tells us how the kidneys and liver are, since their synthesis depends on them, low parameters of albumin are associated with malnutrition, cell inflammation, edema and glomerular damage.

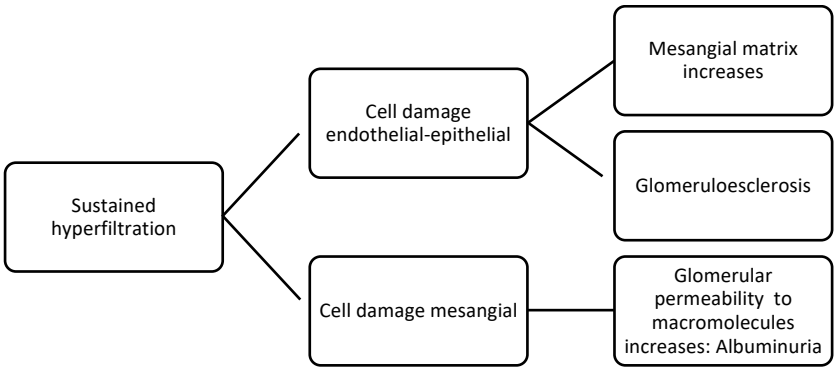


**Figure 6**. Sustained hyperfiltration causes CKD

Advanced glycation end products (AGEs) is also affected in diabetic nephropathy. From these can be obtained residues of proteins, lipids and nucleic acids and their accumulation affects the glomerulus in the renal interstitial, which results in the disease passing from an acute state to a chronic one. AGEs can activate receptors on monocytes-macrophages, endothelial cells, mesangial cells and podocytes (Fierro, 2009). The thickening of the basement membrane in the kidneys begins after the first two years of presenting DM; and after 10 years the thickness will already be doubled (Torres-Viloria et al, 2002). Diabetic nephropathy leads to CKD, as a consequence of elevated GFR, proteinuria and persistent deterioration in the renal structure. It is important to mention that in Mexico the diagnosis of DM is made one year after having developed the disease and in the world, there are 422 millions of people with DM (WHO, 2018).

As previous mentioned, insulin resistance is another factor that contributes to the development of CKD. This condition leads to vascular complications, such as; alterations of the endothelium and inflammation. Endothelial dysfunction causes atherosclerosis, this increases the mortality of cardiovascular events and increases protein catabolism. It has been observed that drugs such as angiotensin-converting enzyme inhibitors and β-blockers reduce insulin resistance, improving glucose uptake and insulin signaling in muscle cells by improving microcirculation (Teta, 2014).

The guidelines of the Kidney Disease Improving Global Outcomes (KDIGO) define CKD as the persistence of kidney damage after three months, with a GFR <60ml / min1.73m$^2$. The TFG allows a classification in five stages (Table 1). Of these, three are asymptomatic stages, which can maintain homeostasis and a healthy quality of life. Stages one through three can be delayed or maintained, but not reversed. It is important to mention that the diagnosis of CKD when carried out, the patient is in a stage three or more. Because of this, prevention is a crucial factor, to decrease the development of the disease in the world. Nutritional therapy and exercise can delay the progress of CKF. Stage four and five are therapeutics, in these the use of drugs is essential, due to the presence of alterations in the metabolism. After stage five, alternative therapies such as HD or peritoneal dialysis (PD) will be needed. Another option is the transplants of a living or cadaveric donor, unfortunately the lack of a culture of organ donation, has as a consequence that this option is not so available (Espinosa-Cuevas, 2016). CKD involves many changes in the

morphology of the kidney, which are permanent, due to this there are alterations in the metabolism. In addition, the evolution of CKD affects other organs of the body, such as the liver in the hepatorenal syndrome, the lung with metabolic alkalosis, and in the bone marrow decreases the production of erythropoietin.

**Table 1.** Renal stages establish by the KDIGO guidelines

| Stage | Alteration | GFR ml/min/1.73m$^2$ |
|---|---|---|
| **1** | Renal lesion with normal or increased glomerular filtration | >90 |
| **2** | Renal lesion with slight decrease of glomerular filtration | 60-89 |
| **3** | Moderate decrease of glomerular filtration | 30-59 |
| **4** | Severe decrease of glomerular filtration | 15-29 |
| **5** | Chronic kidney disease end stage | <15 |

### 3.1 Biomarkers of kidney damage

Currently, in addition to the GFR to assess kidney damage there are other markers that are frequently used to assess damage and are: creatinine, cystatin C, urea, $K^+$, $PO_4^{-3}$, $Na^+$, $Ca^{+2}$, presence of anemia and blood urea nitrogen (BUN).

1) Blood creatinine: This is derived from the metabolism of muscle tissue, being proportional to muscle mass, the greater the muscle depletion, the greater the amount of creatinine in the blood. Creatinine increase when GFR has decreased by 50%. Therefore, creatinine needs advanced tissue damage to observe changes in the parameter. Also influenced by age, weight, race and sex (Espinosa-Sevilla et al., 2013).
2) Cystatin C: is a protein of low molecular weight, inhibitor of protein kinase and is secreted by the proximal tubule in the kidney; when GFR decreases, cystatin C also increases (Espinosa-Sevilla et al., 2013).
3) Urea: it is synthesized in the liver and is the final product of protein metabolism. Once generated, it is excreted in the kidney. It is metabolized at the intestinal level and the ammonium produced is converted into urea. Low molecular weight is filtered at the glomerular level and reabsorbed by 40% at the level of the proximal tubule. Due to, hyper catabolism or tissue destruction can be evaluated (Rodríguez-Puyol et al., 2007).
4) BUN: the BUN is equivalent to the nitrogen ingested less the eliminated nitrogen, present in the urine in the form of urea. It is an indicator of protein metabolism, used for the evaluation of hyper catabolism (Nicolás-Martinuzzi et al., 2011).

### 3.2 Pharmacological treatment in CKD

Due to the etiology of CKD, the use of drugs is necessary as seen previously. The drugs in the patient with CKD can be divided according to the metabolism or the system that needs to be controlled. Also, it must be considered, that most drugs have side effects. On the

other hand, CKD requires the interaction of various drugs to achieve the therapeutic objective. Below, the most used therapies for this disease are listed.

### 3.2.1. Drugs for the control of hypertension

The objective of antihypertensive treatment is to reduce blood pressure, decrease the progression of CKD and reduce cardiovascular risk. Control in the renin-angiotensin system has shown nephroprotective effects in patients with DM (Ros-Ruiz et al., 2012), 80% of patients with CKD and hypertension requires two or more drugs (Méndez-Durán, 2013), of the most used can be mentioned angiotensin receptor antagonists (ARA), the inhibitors of the enzyme converting angiotensin (ACE) and β-blockers.

a) Receptors of angiotensin type II (ARA-II): These are fundamental in the treatment of hypertension and heart failure, when people have intolerance to the ACE or CKD is by proteinuria and diabetic nephropathy. ARAs block the binding of type II angiotensin receptors in smooth muscle tissue, the adrenal gland, the myocardium, and the glomerulus. As a side effect is the increase in blood creatinine and potassium. Type II ARAs have demonstrated their efficacy in controlling blood pressure and reducing urinary excretion of albumin and in turn reducing the progression of CKD. Losartan is a type of ARAs.
b) ACE: ACE inhibitors act on the renin-angiotensin-aldosterone system. Angiotensin I is converted to angiotensin II, this hormone is responsible for vasoconstriction. ACE inhibitors block the effects of angiotensin II, specifically its binding to receptors in the adrenal cortex, resulting in the release of aldosterone. Aldosterone regulates cellular $Na^+$ retention specifically in the collecting tubule. ACE inhibitors reduce blood pressure, but increase the $K^+$ concentration, which is why they are not recommended in the later stages of CKD (Baltatzi et al, 2011). Enalapril, captopril are common drugs of this group.
c) β-blockers (BB). They produce a competitive and reversible blockade of catecholamines mediated through the stimulation of β-adrenergic receptors. BB binds to β-adrenergic receptors and prevents the activation of the signaling pathway Gs.adenilyl cyclase-AMPc-PKA protein by catecholamines, as a consequence it reduces cellular levels of cAMP, and inhibits protein kinase A and phosphorylation of various cellular proteins. BB reduces blood pressure by blocking beta1-adrenergic receptors, this translates into a reduction in plasma renin activity in renal juxtaglomerular cells. Metoprolol is a common β-blocker.

### 3.4.2. Drugs to avoid anemia

Vitamin B12 supplementation is recommended. Most supplements need vitamin B12 to have synergy and perform their expected function.

a) Oral iron supplements. It is recommended to administer it two hours before or one hour after the chelators of phosphorus, in order to increase its absorption at the intestinal level. It should not be eaten in conjunction with legumes, cereals or products with a high content of tannins (Cuevas et al, 2008).

b) Intravenous iron: iron saccharate (Venofer). All active forms have been associated with adverse effects of immunological type. Anaphylactic reactions are very frequent in iron without dextran, such as venofer (Cuevas et al., 2008).
c) Human recombinant erythropoietin (r-HuEPO). The r-HuEPO has two forms, alpha and beta, indicated in the treatment of CKD. The r-HuEPo stimulates erythropoiesis, which in patients with CKD has been affected. The European Best Practice Guidelines (EBPG) states that patients in stages 3-5 must be treated with agents that stimulate erythropoietin, in addition to the addition of oral iron. In patients on replacement therapy the use of r-HuEPO is mandatory, especially in patients on PD (Cabrera-García et al, 2009).

### 3.2.3. Drugs for the control of glucose

a) Biguanide such as Metformin is contraindicated in TFG <30ml / min / 1.73m$^2$, it is recommended to monitor renal function due to the risk of lactic acidosis (Serra-Sansone, 2016).
b) α-glucosidase inhibitors. Acarbosa; they act by decreasing the intestinal absorption of simple carbohydrates and as a consequence a delay in absorption that results in the reduction of postprandial hyperglycemia. It is not recommended in TFG <25ml / min / 1.73m$^2$ (Serra-Sansone, 2016).
c) Glibenclamide stimulates β-pancreatic cells, by binding and inhibiting ATP-sensitive potassium channels. This inhibition causes cellular membrane depolarization and, opens calcium channels, the intracellular calcium increases, and insulin is release (Serra-Sansone, 2016).

## 4. Liver Disease (LD)

### 4.1. Physiology and liver anatomy

Another organ responsible for the elimination and detoxification of the body is the liver, unlike the kidney, the liver has the ability to regenerate. This one is located in the upper right part, below the diaphragm and has an approximate weight of 1400 to 1800 g. The liver is divided into greater right lobe, left lobe, caudate lobe and square lobe. This organ receives blood from the portal vein and the hepatic artery (Figure 7). The anatomy of the liver is important, with it, the origin of the hepatic lesions can be determined (Sibulesky, 2013).

The main functions of the liver are to excrete the products of protein catabolism, it is part of the coagulation, it is fundamental in the digestive, immunological and hematological functions. In the hepatocyte, the glucose can be oxidized into free fatty acids (mitochondria) these are esterified in triglycerides and cholesterol esters (Carillo-Esper and Muciño-Bermejo, 2011). The hepatic lobules are divided into three zones; periportal zone or zone 1, middle zone or zone 2 and pericentral zone or zone 3. The hepatocytes of zone 1; they are responsible for gluconeogenesis, urea synthesis and β-oxidation of fatty acids, while those in zone 3 are responsible for glycolysis and lipogenesis. Hepatocytes have an approximate size between 15 and 25 μm (Delgado-Coello and Mas-Oliva, 2017).

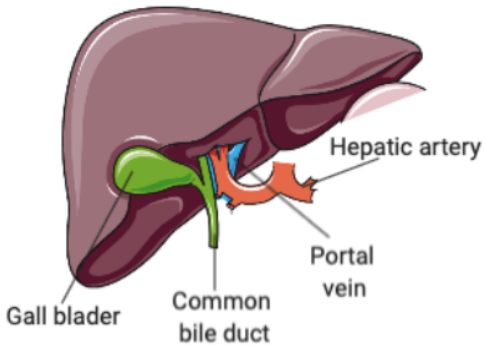


**Figure 7.** Liver anatomy

In summary, the liver has three important functions:

1. Metabolism:
    a) Glucose metabolism.
    b) Lipid metabolism.
    c) Amino acid metabolism.
2. Detoxification and excretion.
3. Immunologic.

### 4.2 Metabolic, detoxification and immunologic functions of the liver

#### 4.2.1 Hepatic metabolism

The liver is responsible for maintaining homeostasis, within the metabolism of glucose and lipids. Glycolysis is the metabolic pathway responsible for oxidizing glucose to pyruvate, that later will be sent to another pathway in order to obtain energy. On the other hand, excess glucose is used to synthesize glycogen, it is worth mentioning that the liver is the organ with the greatest capacity to synthesize glucose, since it can be synthesized from amino acids, pyruvate and lactate (Leoni et al., 2018). The liver also plays an important key role in maintaining adequate levels of glucose. Meanwhile, in the lipid metabolism, triglycerides are hydrolyzed into free fatty acids and then transport to the liver to be oxidize (β-oxidation) and obtain acetyl-CoA or be esterified into glycerol and VLDL are formed. Additionally, it is the only organ that can convert cholesterol into bile acids (Zhuang et al., 2019). Another important metabolic pathway in the liver is the ketogenesis, it is the creation of ketone bodies due to excess of acetyl-CoA, this substrate is used as energy in cases of extreme fasting. The alteration in homeostasis of hepatic metabolism results in various diseases such as: chronic liver disease, alcoholic fatty liver, non-alcoholic fatty liver, hepatic steatosis, insulin resistance, DM and obesity (Sarmiento-Quintero et al., 2016). Each of the metabolic pathways mention is described below:

1. Glucose metabolism (Figure 8): after the consumption of food, carbohydrates ingested are hydrolyzed until glucose molecules are obtained. These are metabolized for energy; the liver has an essential function in maintaining glucose levels for the body (Leoni et al., 2018; McManus and Mitchell, 2014).

a) Glycolysis: this metabolic pathway only consumes about 20-30 % of glucose (inside the liver), the rest is used for the synthesis of glycogen, free fatty acids and ketone bodies. Hepatocytes have a glucose transporter GLUT2 type, which is not insulin dependent, this allows glucose to pass freely inside the cell membrane. Once the glucose is inside the cell, the enzyme glucose kinase, phosphorylates glucose to glucose-6-phosphate this allows this substrate to be used in glycolysis, pentose phosphate pathway and glycogenesis.
b) Glycogenesis: in this metabolic pathway glycogen is obtain. This from glucose-6-phosphate, this pathway is activated by insulin in response to high glucose levels. In postprandial stages, the liver releases these glucose molecules into the bloodstream to provide energy to the erythrocytes.
c) Gluconeogenesis: is the synthesis of glucose from different substrates such as: alanine, lactate and glycerol, the liver can synthesize glucose molecules. This system is only activated, when it is in a prolonged state of fasting.

2. Lipid metabolism (Figure 8): within the metabolism of fatty acids there are two hormones that play an important key role: insulin and glucagon. In the liver, the glycogen after been oxidase is store as free fatty acids or cholesterol. Free fatty acids are transformed into phospholipids or sphingolipids. On the other hand, high levels of acetyl-CoA can result in the synthesis of ketone bodies (Leoni et al., 2018; McManus and Mitchell, 2014).
   a) Synthesis of free fatty acids: as previously mentioned, in the liver the excess of acetyl-CoA is oxidized into free fatty acids. This process is performed in the mitochondria by β- oxidation. Free fatty acids are essential in the body. In addition, they are precursors in the synthesis of bioactive lipids.
   b) Oxidation of fatty acids: during this process, the bonds between carbon atoms α and β are broken down, acetyl-CoA is formed, this process is also known as β-oxidation. β-oxidation occurs mainly within the mitochondria, although it can be performed within peroxisomes. The liver, muscle and myocardium have an affinity with used these compounds as energy.
   c) Ketone bodies: these are produced from the remaining molecules of acetyl-CoA (β-oxidation). In the ketogenesis, acetoacetate, b-hydroxybutyrate and acetone molecules are obtained, this process is carried out within the matrix of hepatic mitochondria. The heart muscle and skeletal muscle use ketone bodies to generate energy.
   d) Synthesis of cholesterol: after eating foods with fatty acids, triglycerides are hydrolyzed by pancreatic lipase into glycerol and two free fatty acids and 2-monoacylglycerol is obtained. This process is carried out in the intestine. Cholesterol esterase hydrolyzes cholesterol esters to free fatty acids and cholesterol. Then, phospholipase A2 digests phospholipids to free fatty acids and lysophospholipid. These molecules are packaged into micelles, in order to be absorbed in the microvilli of the intestine. Cholesterol is an important component within biological membranes and, it is a precursor to sterols, hormones and bile acids.

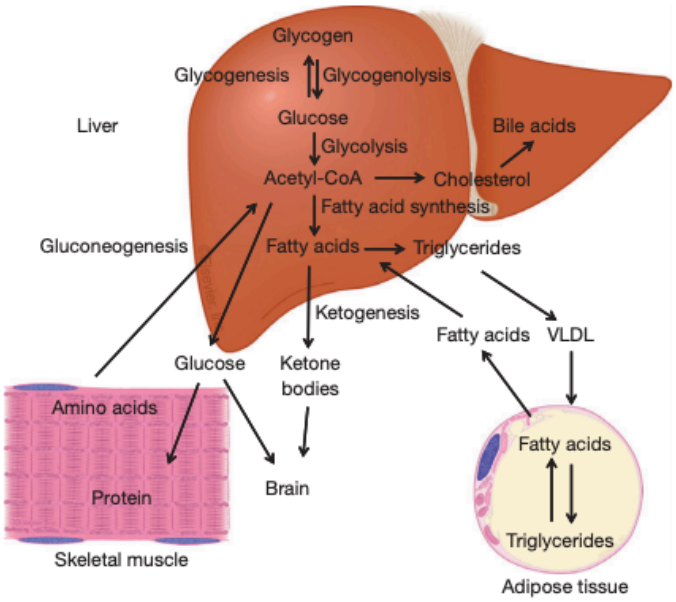


**Figure 8.** Liver metabolism, glucose and lipids pathways (Taken from McManus and Mitchell, 2014).

3. Amino acid metabolism and urea cycle: the liver plays a key role in maintaining the levels of amino acids. After, eating food most of the amino acids are absorbed for protein synthesis in the liver. Then, the amino acids enter to the hepatocyte through the plasma. Alanine and glycine are transported by a membrane protein that acts on sodium coupled transport. The aromatic amino acids are synthesized by the liver and, this regulates the amount of amino acids that passes into the circulation. Meanwhile, branched chain amino acids cannot be synthesized by the liver and are used in the muscle. The excess of amino acids in the muscle produces alanine and glutamine. These are transported to the liver where they are used in gluconeogenesis. Furthermore, the excess of amino acids are degraded by the urea cycle, where nitrogen is converted to urea and, then excreted by the kidneys (Carretero, 2004; Ah-Mew, Pappa & Gropman, 2015; Barmore & Stone, 2019).

   For this reason, another important pathway in the liver is the Urea Cycle. This is responsible for the elimination of ammoniac. The urea cycle can only be performed in the mitochondria of the liver (Figure 9). Ammonia is a toxic compound that is obtained from the synthesis of amino acids. This reaction is done by adding three amino groups. In addition, six enzymes are required: N-acetyl-glutamate synthetase, carbamoyl phosphate synthetase, ornithine transcarbamoylase, argininosuccinate synthetase, argininosuccinate lyase and arginase. The three additions are described below (Ah-Mew, Pappa & Gropman; 2015 Barmore & Stone, 2019):

a. The first amino group that enters the cycle, comes from free intramithocnrial ammoniac. Then, ammonia and the bicarbonate is used to produce carbamoyl phosphate; this reaction is ATP dependent and catalyzed by carbamoyl phosphate synthetase I. This reaction requires and activator N-acetyl-glutamate.
b. Citrulline is formed and released to cytoplasm. Through the enzyme ornithine transcarbamoylase. The second amino group comes from aspartate, and arginosuccinate is formed. This reaction is catalyzed by argininosuccinate synthetase.
c. Argininosuccinate is hydrolyzed by argininosuccinate lyase. And arginine and fumarate are formed. Finally, the fumarate enters the Krebs cycle and through arginase urea and ornithine are formed. In the form of urea, can be excreted by the kidney.

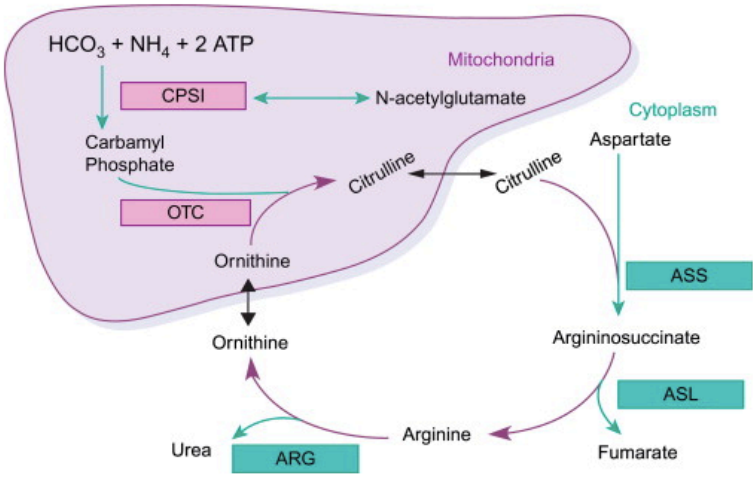


**Figure 9.** Urea cycle (Taken from Ah-Mew, Pappa & Gropman, 2015).

### 4.2.2 Detoxification of the liver

In order to excrete non-soluble drugs, xenobiotics of metabolites, these has to be metabolized in the liver. Both drugs and fat-soluble chemicals are transported into plasma through proteins to the liver, in order to convert them into water-soluble molecules and be able to eliminate by the kidney (McManus and Mitchell, 2014). These enzymes are responsible for oxidizing, reducing, hydrolyzing or methylation process. This is achieved with three different phases:

1. Drug oxidation; the CYP monooxygenases contain oxidation and reduction enzymes. These reactions take place in the cytochrome P450. Monooxygenases metabolize substrates by incorporating an oxygen atom of $O_2$ molecules and form -OH.
2. Conjugation reaction: includes sulfation, methylation, and glucuronidation, these reaction in order to increase the solubility of the metabolites.

3. Drug transporters: these, takes the metabolites into the membranes of hepatocytes, kidney and intestine cells for excretion. The liver can also excrete some hormones such as: thyroxine, estrogen, cortisol and aldosterone.

### 4.2.3 Importance of liver immune system

Immunological: As mentioned before, hepatocytes constitute approximately two thirds of the total liver cells, the rest is divided into endothelial cells, Kupffer cells, lymphocytes, bile cells and astrocytes. Kupffer cells are the largest group of fixed macrophages in the body and are derived from circulating monocytes and, when hepatocytes enter into an apoptotic state the Kupffer cells are responsible for phagocytizing them (Aguilar, 2009; McManus and Mitchell, 2014).

## 4.3 Non-alcoholic fatty liver disease (NAFLD)

Liver pathophysiology is very wide and is a mixture of several factors. Liver diseases can depend on several factors such as: Infectious (hepatitis), metabolic (fatty liver) and dietary (liver cirrhosis) (Zhuang et al., 2019). For the purposes of this chapter, we will be focusing on the non-alcoholic fatty liver disease (NAFLD). This is defined as the presence of fatty acids in at least 5 % of hepatocytes and that there are no other diseases such as hepatitis or inborn errors of metabolism. In Mexico, a prevalence of 17.1% has been reported, while in the US of 20-30 % and, a worldwide prevalence is 25.2 % (WHO, 2016). In addition, an increase in prevalence has been observed according to age (more in adult people). The risk factors for developing NAFLD are weight gain, metabolic syndrome and insulin resistance. These are addressed below.

### 4.3.1 Pathophysiology of NAFLD

Fatty liver disease or NAFLD is derived from overweight and obesity, it has been observed that pathologies such as insulin resistance and metabolic syndrome aggravate this disease, this can be seen in Figure 10 (Bernal-Reyes, 2015; Zhuang et al., 2019). In Mexico, the leading cause of liver disease in men is liver cirrhosis that results from alcoholism, but in women it is fatty liver and is associated with excess weight and lifestyle. It has been observed that most patients with NAFLD have these types of diets (high in saturated fatty acids), if these fatty acids are not oxidized, they are stored into cytoplasmic deposits, the excess in these deposits promotes cell lipotoxicity and mitochondrial dysfunction.

Meanwhile, free fatty acids that are in circulation perpetuate insulin resistance (Delgado-Cortes et al., 2018). The excess of free fatty acids leads to the accumulation of triglycerides inside the liver, and also causes structural changes in the mitochondria of the hepatocyte and produces oxidative stress. Furthermore, free fatty acids can induce some microsomal lipogenases in the cytochrome p-450 leading to the production of free radicals that are hepatotoxic. Another factor that also influences NAFLD is intestinal dysbiosis, it contributes to the release of endotoxins into the portal circulation, which affects the liver's immune system, promoting and increasing cellular inflammation (Carillo-Esper and Muciño-Bermejo, 2011).

In order to diagnosis NAFLD is necessary a liver biopsy. Afterwards, the histopathology slice is evaluated and graded (histological activity). In 2005, the Clinical Research Network and the National Institute of Diabetes, Digestive and Kidney Disease published a histological scoring system for NAFLD this can be observed in Table 2, it scores both adult and pediatric NAFLD biopsies (Brunt et al., 1999; Brunt and Tiniakos, 2010).

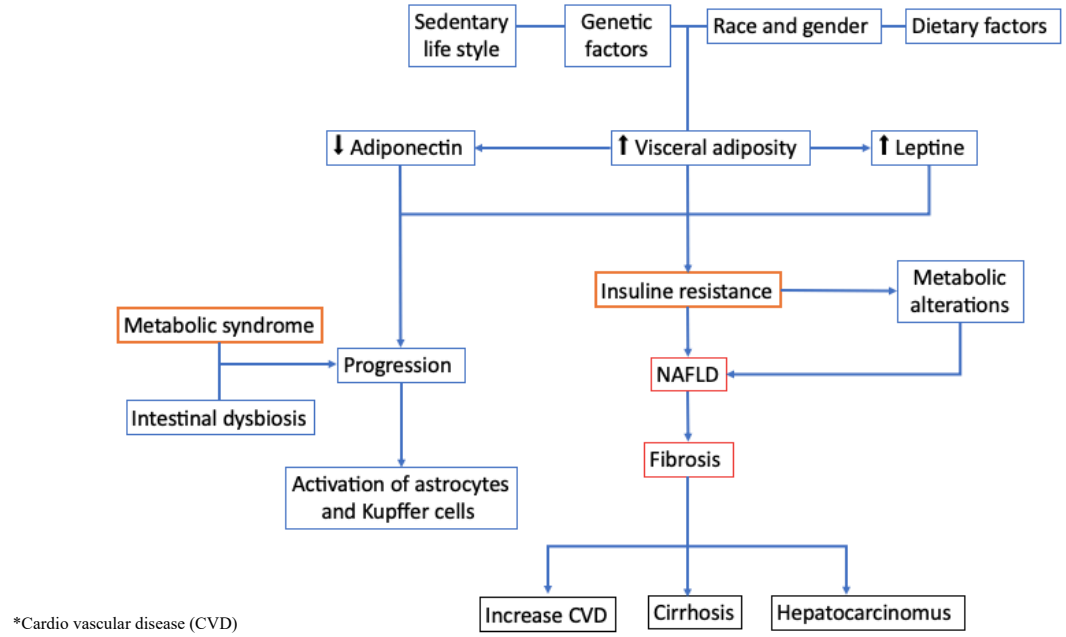


**Figure 10**. Basic pathophysiology of NAFLD (Modified from Delgado-Cortes et al., (2018)).

The system also evaluates fibrosis with the method proposed by Brunt et al., (1999), fibrosis is divided into four areas:

- Fibrosis stage 1: Zone 3 peridinisoidal fibrosis, focal or extensive.
- Fibrosis stage 2: Zone 3 with focal or extensive periportal fibrosis.
- Fibrosis stage 3: bridging fibrosis.
- Fibrosis stage 4: Cirrhosis, probable or definite.

**Table 2**. Grading activity and staging fibrosis in NAFLD according to Brunt (Brunt and Tiniakos, 2010).

| **Stage** | **Steatosis**<br>**1: < 33 %**<br>**2: 33 – 66 %**<br>**3: > 66 %** | **Ballooning (zonal location and severity recorded)** | **Inflammation** | |
|---|---|---|---|---|
| | | | **L-lobular**<br>**0: absent**<br>**1: < 2**<br>**2: 2-4**<br>**3: >4 foci** | **P-portal**<br>**0: absent**<br>**1: mild**<br>**2: moderate**<br>**3: severe** |
| **Grade 1 (mild)** | 1-2 | Minimal, zone 3 | L = 1-2 | P = 0-1 |
| **Grade 2 (moderate)** | 2-3 | Present, zone 3 | L = 1-2 | P = 1-2 |
| **Grade 3 (Severe)** | 2-3 | Marked, predominantly zone 3 | L = 3 | P = 1-2 |

Another important complication of LD is the hepatorenal syndrome (HRS), a conjunction with renal disease. The pathophysiology of HRS is complex, but it can be related to 3 areas: the splanchnic bed, the sympathetic nervous system (SNS) and the cardiac system. In addition, there are three factors that favor the development of SHR: cirrhosis, ascites and portal hypertension. The increase in portal vein pressure activates the release of endogenous vasodilators such as nitric oxide (NO), carbon monoxide (CO) and cannabinoids, increasing local plasma volume and rapidly decreasing mean arterial blood pressure. On the other hand, the release of vasopressin is necessary, activating the renin-angiotensin-aldosterone system, decreasing the GFR and retaining water and $Na^+$, this would explain the development of ascites in patients with SHR. The consequence in the renal anatomy before the activation of the vasoconstrictors is the vasodilator stimulus that affects the tubular and hemodynamic function. Severe renal vasoconstriction causes a decrease in GFR. Persistent changes in circulation decrease cardiac output, with an increase in plasma renin, favoring the appearance of hypovolemia, renal hypoperfusion and the development of HRS (Ospina and Restrepo, 2015).

### 4.1 Biomarkers of liver damage

In addition to the guideline of Brunt and Tiniakos, (2010) shown in Table 2, another way to diagnose liver disease and specifically NAFLD is with hepatic biomarkers. The alterations in serum aminotransferases can shown hepatic injury. Serum aminotransferases: are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). ALT is found in the liver parenchyma and AST is found in the liver, heart, skeletal muscle, pancreas and lung. So, the AST is not as specific as the ALT. The elevation in serum levels usually indicates injury or necrosis in the hepatocytes (Cortés and Montoro, 2010).

1) Alkaline phosphatase (ALP) and gamma-glutamyl-transpeptidase (GGT): GGT is an enzyme found in the hepatocyte and in the biliary epithelium. ALP is found in the liver, bone, intestine and placenta. An elevation of both is a sign of hepatic cholestasis (Cortés and Montoro, 2010).
2) Bilirubin: is a product of the degradation of the catabolism of hemoglobin. It is metabolized in the smooth endoplasmic reticulum of the hepatocyte, by glucuronyl-transferase (Cortés and Montoro, 2010).

### 4.2 Pharmacological treatment in LD

The main treatment in NAFLD is weight loss. There is no specific medication to treat NAFLD. However, studies suggest that some medications such as: hypoglycemic, vasoconstrictors and hypolipemic can help through NAFLD. Hypoglycemic medication was addressed previously in the renal section.

#### 4.2.1. Drugs for the control in liver disease

The treatment with vasoconstrictors and albumin is one of the first options to choose. The goal of treatment is to produce vasoconstriction in the splenic vascular bed and reduce hypovolemia.

a) Alpha adrenergic agonists: norepinephrine, midrodine. Norepinephrine is a catecholamine that acts on adrenergic receptors. Midodrine acts as a selective agonist of peripheral alpha-1 receptors, generating vasoconstriction and increased blood pressure (Ospina and Restrepo, 2015).
b) Somastostin analogues: Octreotide, an octapeptide analogous to somastatin with potent vasoconstrictor action on the splenic vasculature. It acts as an inhibitor of the secretion of peptides synthesized by the gastro-endocrine-pancreatic endocrine system, having the effect of decreasing splenic blood flow (Ospina and Restrepo, 2015).
c) Vasopressin analogues: ornipressin, terlipressin. Ornipressin has shown benefits in SHR, but due to its ischemic effects such as arrhythmias, myocardial ischemia and cutaneous necrosis, it has been abandoned. Terlipressin is a synthetic derivative of vasopressin, which has a dominant action on V1 receptors, with a potent vasoconstrictor effect. The administration of volume expanders such as albumin improve the effect of vasoconstrictors (Ospina and Restrepo, 2015).

### 4.2.1. Drugs for the control of dyslipidemias

a) Statins: Atorvastatin, simvastatin, pravastatin, rosuvastatin, pitavastatin and fluvastatin. They reversibly inhibit cholesterol synthesis and are effective in reducing low density lipoprotein (LDL) and moderately reducing triglyceridemia. Atorvastatin and fluvostatin do not require adjustment to renal function by GFR, being the statins of choice in CKD. Dyslipidemia can cause glomerulosclerosis, the use of statins reduces proteinuria and helps to preserve renal function (Jalill et al, 2017).

## 5. Alternative medicine to the use of drugs

The plants have been used for years to treat diseases. Rigorous safety and efficacy studies are necessary before the prescription of plants for human use. Herbal remedies can cause toxicity in the organism, this is why doses are important (Vivekanand, 2010). Nephrotoxicity is a very common factor in the inappropriate consumption of herbal remedies, because the kidney is responsible for eliminating toxins in the body as mentioned before. The benefits of using plants with nephroprotective effects are poorly understood, but it is estimated that 1 in 5 patients consumes herbal supplements (Cooke, 2004). The use of herbal medicines can be prescribed in CKD and LD, but it is necessary to know the mechanism of action of the plant, these to avoid nephrotoxicity and hepatotoxicity (Luyckx, 2012).

In Mexico the use of plants as an alternative has been used since pre-Hispanic times. At present, the hepatoprotective effect of some plants has been studied, being the most studied *Silybum marianum* (milk thistle), *Rosmarinus officinalis* (rosemary), *Synara scolymus* (artichoke) and *Taraxacum officinale* (dandelion), offering protection against hepatitis, jaundice, fatty liver and cirrhosis. Because of this, phytotherapy represents an alternative to the complications of LD and CKD (Beltrán et al., 2005; Favari et al., 2013).

**6. Chaya (*Cnidoscolus aconitifolius*)**

Chaya is a shrub cultivated in the Mayan region of Guatemala, Belize, southeastern Mexico, parts of Honduras and can be found in Nigeria. It belongs to the family Euphorbiaceae and the genus *Cnidoscolus*, it is composed of 50 species of which 20 are endemic in Mexico (Ross-Ibarra and Molina-Cruz, 2002).

Botanical classification:

- Scientific name: *Cnidoscolus aconitifolius*.
- Kingdom: plant.
- Subrein: vascular plants.
- Category: species
- Division: flowering plant, angiosperms.
- Classification: Dicotyledons.
- Subclass: Rosidea.
- Family: Euphorbiaceae.
- Subfamily: Crotonoideae.
- Gender: *Cnidoscolus*.
- Species: *aconitifolius*.
- Common name: Chaya.

Chaya (*C. aconitifolius*) is a perennial shrub that grows up to six meters in height. It has alternately palmately lobed leaves, milky sap and small white flowers that are in clusters, its leaves are large; up to 32 cm long and 30 cm wide, with petioles 28 cm long, three toxic varieties are known and only one is edible (Ross-Ibarra, 2003). The four cultivated varieties of chaya have been established; estrella, picuda, chayamansa and redonda, these based on the shape of the leaves (Ross-Ibarra and Molina-Cruz, 2002). In Figure 11 these four forms can be seen. Traditionally known as chaya in the Mexican southeast, in Mayan language with the name of "Ts'its'ik-chay" or "Xts'ats, tsats", in other countries *C. aconitifolius* is known by different names such as: "Tree spinach" in the United States and "Efo Jerusalem" or "Efo Iyanna Ipaja" in Nigeria (Ross-Ibarra, 2003; Markus et al., 2016).

The mature leaves of *C. aconitifolius* are broad and have three or more lobes, the growth of the plant is rapid, the leaves are edible (Chayamansa variety), and the shoots can be produced in a period of 8 to 10 weeks. Chaya has very small urticating hairs and located on the petiole and the abaxial margin of the blade, rarely produces fruits or seeds and lacks pollen production (Ross-Ibarra, 2003). It is cultivated in sub-humid warm climates, it can tolerate heavy rains and drought, is a backyard (solar) plant, that can be found in family gardens. Its propagation is easy and is a species resistant to pests, although the *Corythucha spp* (insect) affects the leaf and *Puccinia spp* (fungus) attacks the stem (Pérez-González, 2016).

Chayamasa has been cultivated since pre-Hispanic times. In Mexico its use as an edible, and medicinal plant, it is also part of the traditional foods of the southeast part of the

country since the Mayan culture. In natural medicine was used to combat skin problems, eye problems, kidney stones and obesity. Its antibacterial, hypoglycemic effect as a co-adjuvant in anemia has been seen in several studies (Akachukwu et al., 2014).


**Figure 11**. The four varieties of chaya (Cifuentes et al., 2012)

### 6.1 Chemical and nutritional composition of *Cnidoscolus aconitifolius*

The presence of protein and vitamins such as thiamine, niacin, riboflavin, retinol, beta carotene and ascorbic acid has been found in leaves and stems. The leaves of *C. aconitifolius* contains minerals such as: $Ca^{+2}$, iron ($Fe^{+3}$), $PO_4^{-3}$, $K^{+}$, $Mg^{+2}$, $Na^{+}$, manganese ($Mn^{+2}$), zinc ($Zn^{2+}$) and copper ($Cu^{+}$). The amino acids that can be found are threonine, lysine, arginine, histidine, aspartame, proline, serine, alanine, glutamic acid and glycine, giving a high nutritional value to the plant (Markus et al., 2016., Aye, 2012.) Pérez-González et al., 2016). In Table 3, the nutritional composition of chaya leaves are shown. Chikezie et al., (2016), reports the composition of vitamins in fresh leaves per 100 g of vitamin A 13.109 mg, vitamin B6 1.34 mg, vitamin B9 1.06 mg, vitamin B12 0.13 mg and vitamin C 142.11 mg.

**Table 3**. Proximate (g / 100 g) and mineral (mg / 100 g) composition of cooked and sun-dried leaves of *C. aconitifolius* (Modified from Aye, 2012; Chikezie et al., 2016)

| Parameter | Cooked | Dry with sunlight |
|---|---|---|
| **Ashes** | 12.70 ± 0.02 | 13.68 ± 0.03 |
| **Raw protein** | 17.18 ± 1.16 | 19.20 ± 0.60 |
| **Raw fiber** | 9.26 ± 0.02 | 8.54 ± 0.01 |
| **$Na^{+}$** | 45.21 | 68.52 |
| **$K^{+}$** | 69.22 | 105.5 |
| **$Ca^{+2}$** | 34.79 | 43.22 |
| **$PO_4^{-3}$** | 96.59 | 126.56 |
| **$Mg^{+2}$** | 20.31 | 22.51 |
| **$Zn^{+2}$** | 36.03 | 47.84 |
| **$Fe^{+3}$** | 39.41 | 48.58 |
| **$Cu^{+2}$** | 2.03 | 2.38 |
| **$Mn^{+2}$** | 0.06 | 0.39 |

The process of cooking the leaves of chayamansa affects the mineral composition of the plant, this is why other methods such as drying are preferred. Minerals exert vital functions in the organism, such as mineral metabolism, red cell maturation, muscle contraction and

controls blood pressure (Markus et al, 2016). The phytochemical studies carried out in the extract of *C. aconitifolius* show the presence of alkaloids, saponins, phenols, tannins, flavonoids and triterpenes, this can be seen in Table 4. For its part, Chukwunonye et al., (2017) reports presence of tannins, lignin, fatty acids, protein, calcium carbonate and calcium oxalate. Otitoju et al., (2016) reported the presence of alkaloids, anthocyanins, carotenoids, flavonoids, saponins and tannins.

Fresh chaya leaves are toxic, due to their cyanogenic glycosides content, that can be release by hydrolysis of the hydrocyanic acid (HCN), the cyanide molecules are toxic to human metabolism (Kuti and Konoru, 2006). Among its cyanogenic glycosides are linamarin, tannins and lignin polymers. The cyanogenic glycosides are eliminated at temperatures between 40 ° and 60 ° C, because the cyanide is a gas molecule and with the heat is evaporated, this is why it is important to consume the leaves of *C. aconitifolius* once they have undergone a cooking process. If cyanogenic glycosides are not eliminated, cyanide accumulates in the liver and it cannot be metabolized, causing hepatotoxicity.

**Table 4**. Phytochemical composition of leaf extracts of *C. aconitifolius* (Awoyinka et al., 2007; Akachukwu et al., 2014; Hamid et al., 2016)

| Phytochemicals | Aqueous extract | Ethanolic extract | Hexanoic extract | Ethyl acetate extract |
|---|---|---|---|---|
| **Saponin** | + | ++ | - | + |
| **Tannins** | + | + | - | - |
| **Alkaloids** | - | + | - | - |
| **Phenol** | + | | ND | - |
| **Flavonoids** | + | - | + | - |
| **Anthraquinone** | + | - | - | + |
| **Cardiac glycoside** | + | | + | - |
| **Steroids** | - | | + | + |
| **Terpenoids** | + | | + | + |

+ presence, - absence, ND no data.

### 6.2 Bioavailability of phytochemicals

The bioavailability of a drug or phytochemical is defined as the rate to which the active ingredient is absorbed and is available at a specific site so the drug or phytochemical may act (FDA, 2010). There are several stages that are involved in the bioavailability, these are; liberation, absorption, distribution, metabolism and elimination (LADME). Bioactive food compounds need to be bioavailable to exert any biological potential effects. Numerous factors may influence the bioavailability such as; bioaccesibility, transporters, molecular structures and enzymes (Rein et al., 2013). These factors will be addressed below and in the Figure 12:

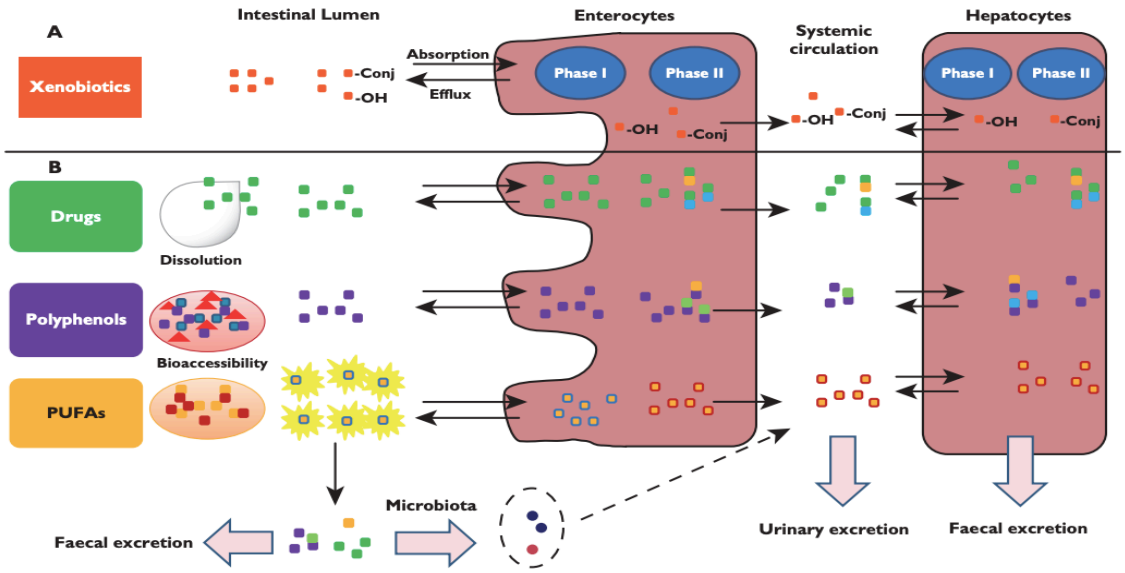


**Figure 12.** Bioavailability of phytochemicals through the intestinal lumen (Taken Rein et al., 2013).

a) Bioaccessibility; is the fraction of a compound that is released from the food matrix in the gastrointestinal lumen and they could be available for intestinal absorption. This process can be altered by the digestion and the synergism and antagonism of different components and digestive enzymes. The temperature and pH are other factors that can have influence on it. Once, they are released from the food matrix they become bioaccessible (Rein et al., 2013).

b) Solubility; this will depend if the compounds are lipophilic or hydrophilic, because it results in different absorption mechanism. The lipophilic compounds pass unaltered through the intestinal wall due to the similarities in the structure. The uptake of lipids is by the enterocyte, it takes place through passive diffusion and facilitated diffusion via transporters, lipids are store in the liver, unlike the hydrophilic compounds they are not readily excreted.

   Hydrophilic compounds like phenols have a more simplistic mechanism of absorption, it takes place at the brush border of small intestine epithelial cells, this liberates the aglycone. After that, the aglycone enters the enterocyte and the can be methylated or glucuronidated. There are some compounds that escapes this face like flavonoids, they pass directly through hepatocytes, via the portal vein.

c) Structure; the molecular structure and the isomeric configuration are important because they affect the absorption process. High molecular weight compounds do not pass through the intestinal cells, they have to be broken down first.

d) Transport mechanisms; the absorption will depend of the mechanism inside the lumen, these could be passive diffusion, facilitated diffusion and active transport. Some phytochemicals do not have the physicochemical properties for passive diffusion mechanism, there for trans-membrane transporters are needed. Intestinal transport systems have different selectivity, and this will depend on the bioactive compound.

### 6.3 Biological potential of *C. aconitifolius*

The potential biological effect of a plant is through its content of secondary metabolites, these can be used to treat diseases and may be used as new drugs, avoiding side effects and the high cost of chemically synthesized drugs. The activities with potential biological effect are broad such as; antioxidant effect, hypoglycemic, hypotensive, hypolipidemic or glucose regulation. In the following sections, these effects will be described.

The antioxidant activity is fundamental for life, which is why it is necessary to obtain foods that offer an antioxidant capacity, which protects the cell from the oxidation of free radicals. The antioxidant potential of *C. aconitifolius* has been studied by several researchers. Kuti and Konoru, (2004) report that antioxidant levels are higher in the raw leaf compared to the cooked leaf. On the other hand, Ogunlade et al., (2009), report that chaya can reduce Fe (III) to Fe (II), in addition to containing the most active form of vitamin E, $\alpha$-tocopherol an important antioxidant. Vitamin E plays an important role in the endocrine and nervous system, since it has a modulating action on hormonal control, in addition to participating in the normal development of the nerves.

The high content of $Fe^{3+}$ in the leaves of *C. aconitifolius*, suggests the anti-anemic potential, on the other hand the content of vitamin C in the leaves, improves the availability of $Fe^{3+}$, it helps transport oxygen (O-) and metallo-enzymes, present in oxidative phosphorylation (Markus et al., 2016). In anemic rats induced with cyclophosphamide, it was observed that the intake of *C. aconitifolius* for fourteen days improves the concentrations in the hemoglobin and the erythrocyte count, in comparison with the control group (Eze-Eme et al., 2017). The authors suggest an improvement in cellular oxygenation and the possible protective effect in the synthesis of EPO, reducing the osmotic fragility in the anemia associated with PEW. For its part, Emelike et al., (2015), reported a significant increase in weight in rats, associating it with the high nutritional level of the leaves of *C. aconitifolius* that increases appetite and increases dietary intake.

García-Rodríguez et al., (2013) reported a low inflammatory activity in the murine model, indicating a low concentration of flavonoids in the phenolic compounds. Flavonoids are associated with decreased edema in cellular inflammation (García-Rodríguez et al., 2013). Adeniran et al., (2012) reported the antimicrobial potential of ethanol extracts of *C. aconitifolius*, by inhibiting Gram positive and Gram-negative bacteria.

Folaranmi-Olaniyan et al., (2017) reported the lipid-lowering activity of the extract of *C. aconitifolius* in rabbits, this reduces the levels of total cholesterol, LDL and triglycerides, by consuming the extract of *C. aconitifolius* for seven days. The content of fiber in the leaves of *C. aconitifolius* modifies the plasma concentration of lipoproteins and their distribution and composition in the organism. Markus et al., (2016), relates the content of saponins in extracts of *C. aconitifolius* and its role in reducing total cholesterol, in addition to its hypotensive and cardioprotective power.

### 6.4 Potential nephroprotective and hepatoprotective effect of *C. aconitifolius*

Studies conducted with aqueous, ethanolic, methanolic and ethyl acetate extracts of *C. aconitifolius* reported relevant biological activities such as anti-inflammatory, hypoglycemic and hypotensive effects. Aqueous and ethanolic extracts have obtained greater benefits with the extraction of vitamins, minerals, amino acids and phenolic compounds (Folaranmi-Olaniyan et al., 2017; Nath-Roy et al., 2016; García-Rodríguez et al., 2013; Eze -Eme et al., 2017). Other studies have reported biological effects such as kidney and liver protection in the intake of cooked or dried leaf of *C. aconitifolius* (Oyagbemi et al., 2010 and 2013).

Biochemical assessment is needed to recommend an extract of any plant. Because of this, Orji et al., (2016) reported how liver damage with lead acetate ($Pb(C_2H_3O_2)2$), can improve with an ethanolic extract of *C. aconitifolius.* The above, was performed with albino rats, in four different groups; a) control group, b) lead acetate treated group, c) 250 mg/kg of ethanolic extract plus lead acetate, d) 500 mg/kg of ethanolic extract plus lead acetate. The aim of the study was to show the protective effect of *C. aconitifolius* leaves. After, 14 days an improvement in the levels of AST, ALP, ALT, albumin and total proteins, when administering ethanolic extract of *C. aconitifolius* (250 and 500 mg/kg). The authors concluded that the ethanolic extract of *C. aconitifolius* can be a powerful protector in the lead-induced liver damage. This by regulating the alterations in hepatic enzymes and protect the cell membrane permeability or avoid hepatic cell rupture. Overall, the decrease in ALP serum levels indicates that there is no activity associated to necrosis (liver and kidney). Another study about induced hepatotoxicity is reported by Oyagbemi and Azeez (2010), in order to explore the possible protective effect of *C. aconitifolius* aqueous extract on carbon tetrachloride induced hepatotoxicity. The animals were divided in six groups: 1) control, 2-4) administration of 1.25 ml/kg carbon tetrachloride, 3-6) were pre-treated with aqueous extract at 100, 250, 500 and 750 mg/kg respectively. After 10 days of treatment, the animals were sacrificed, examples of ALP, AST, ALT, albumin and total protein were taken. Animals treated with *C. aconitifolius* aqueous extract had lower levels of ALT. The authors conclude that *C. aconitifolius* antagonized $CCl_4$ induced acute hepatotoxicity as evidenced by restoring of liver function.

Oyagbemi and Odetola, (2010) reported the protective effects of the ethanolic extract of *C. aconitifolius* in rats with hepatic damage induced with paracetamol, having as parameters ALT, ALP and AST, urea nitrogen in blood, TC, low density cholesterol (LDL), high density cholesterol (HDL) and total proteins. Three preventive doses of the extract (100, 500 and 1000 mg/kg) were used for seven days, the eighth and ninth day the damage was induced with paracetamol. The authors reported that the groups with pre-treatment of the extract, significantly decreased the levels of ALT, ALP, AST, LDL and blood urea nitrogen.

In order to evaluate kidney and liver function and to observed toxicity as well, Adekomi et al., (2011) fed albino Sprague Dawley rats with an aqueous extracts of *C. aconitifolius* with three different doses (100, 200 and 300 mg/kg) for a period of 14 days. The extract was administered orally once daily with plastic syringes attached to metal oropharyngeal

cannula. At the end of the study, the animals were sacrificed and laparotomy was performed to obtain kidney and liver. Nephrons were obtained with good blood supply and without histological involvement, tubules and renal parenchyma had no distortion and have normal histological function. In general, no gross alterations were observed in the morphology of the kidney or liver. In addition, an increase in the weight of the rats was observed, associating the extract with its high nutritional value. Several studies have shown the different micronutrients that chaya leaves are beneficial to animals, and whets the appetite (Ramos-Gómez et al., 2016, Kuri-García et al., 2017, Orji et al., 2016). In conclusion, many herbal preparations can exhibit renal tubular necrosis showing interstitial fibrosis and severe tubular loss, nevertheless the extracts of *C. aconitifolius* leaves does not have this toxicity.

Afterwards, Akachukwu et al., (2014) reported toxicological effects in liver and kidney after the administration of aqueous leaf extract of *C. aconitifolius* in male Wistar rats. The animals were grouped into four groups of five with different dose of extract (100, 200 and 400 mg/kg) for 28 days, after this period of time a blood test was collected through cardiac puncture and, the kidney and liver were removed for histological analysis. Histopathological examination showed that nephrons and hepatocyte have no damaged or any pathological lesions. There were no significant ($p > 0.05$) effects on the globulin, serum total protein, total and conjugated bilirubin, ALP, AST, ALT, creatinine and urea.

Another mechanism to evaluate that causes hepatic and renal damaged, is protein energy malnutrition. For this reason, Oyagbemi et al., (2013) performed a model of energy malnutrition in male Wistar albino rats, these by feeding the animals with a low protein diet for two weeks, by decreasing the production of protein in the body, liver and kidney damage was induced. The authors took elevations in ALT, ALP, and AST as a reference for liver damage, and increased serum creatinine, albumin, phosphate ($PO_4^{-3}$), and $K^+$ as renal damage. Furthermore, different doses were mixed of *C. aconitifolius* leaf (10% and 20%) with the pellets, for four weeks. The results showed a decreased of ALP, AST, albumin and reduced of the markers of renal damage such as $Na^+$, chlorine, creatinine and urea. The authors concluded that *C. aconitifolius* leaves have a protective role against renal damage and hepatic injury cause by protein energy malnutrition.

Adaramoye and Aluko, (2010) reported the effects of the methanolic extract of *C. aconitifolius* in renal dysfunction induced by chronic ethanol administration. Male Wistar albino rats were distributed into seven groups of six animals. First, a pretreated group with Kolaviron (antioxidant pharmacological treatment) and methanolic extract of *C. aconitifolius* was administered for two weeks. Afterwards, renal injury was induced with chronic ethanol administration for eight weeks. Another group was treated with Kolaviron and the methanolic extract of *C. aconitifolius* for eight weeks in two different doses (100 and 200 mg/kg). The administration of chronic ethanol caused the elevation of serum urea, creatinine, urinary glucose, gamma-glutamyl transferase and total protein, while creatinine clereance decreased. The methanolic extract of *C. aconitifolius* attenuated the increased in serum of gamma-glytamyltransferase, urea, creatinine and, also enhanced the

antioxidant activity by increasing the levels of catalase, superoxide dismutase and glutathione. The protective effect is due to the reduction in oxidative stress and, also the association of the extract as a coadjuvant in the treatment of kidney damage, decreased the damage by lipid peroxidation, improving the oxidation markers and the markers of renal damage (Adaramoye and Aluko, 2010). Another mechanism to protect kidney and liver integrity is hypoglycemic effect. These by decreasing blood glucose levels and avoid renal glycosuria and the formation of free fatty acids in the liver (Leoni et al., 2018; Kalantar et al., 2018). *C. aconitifolius* have reported to decreased glucose in different studies (Nath-Roy et al., 2016; Ramos-Gomez et al., 2016; Ajiboye et al., 2018). The hypoglycemic and antioxidant effect was observed in the study by Ajiboye et al., 2018, with the ethyl acetate extract of *C. aconitifolius* leaves. The authors reported the inhibition of $IC_{50}$ of α-amylase and α-glucosidase of 13.85 and 18.98 µg/mL *in vitro*, concluding that *C. aconitifolius* acts on intestinal enzymes and delays the absorption of glucose. In the antioxidant evaluation with ABTS the $IC_{50}$ was 14.14 µg/mL. The inhibition of amylase and glucosidase at the intestinal level is an important biomarker, to delay the absorption of carbohydrates and avoid elevations in blood glucose. By lowering blood glucose, the excess synthesis of insulin by the pancreas is avoided, this creates a protective effect.

Nath-Roy et al., (2016) evaluated the possible hypoglycemic effect of *C. aconitifolius* ethanolic extract in diabetic mice. The animals were induced to diabetes with a single dose of streptozotocin (60 mg/kg). Furthermore, the authors reported phytochemical screening and it revealed highly levels of alkaloids and flavonoids, crude fiber and, few tannins. After, 28 days of administering ethanolic extract of *C. aconitifolius* in three different doses (100, 150 and 200 mg/kg), a dose dependent reduction in blood glucose levels (23.76 %) was found after intraperitoneally administration of *C. aconitifolius* extract. The authors concluded that *C. aconitifolius* have the ability to reduced glucose blood levels in diabetic mice and, suggesting that bioactive compounds are responsible for these effects. Another study in diabetic rats induced with streptozotocin, was performed by Ramos-Gomez et al., (2016), the aim of the study was to investigate the antidiabetic potential of aqueous *C. aconitifolius* extract. Furthermore, phytochemicals in the shrub were identified and quantified including flavonoids, phenolic acids, saponins and alkaloids. Additionally, antioxidant capacity and digestive enzymes inhibitory was performed, the results expressed as $IC_{50}$ DPPH, ABTS, $\alpha$-amylase and $\alpha$-glucosidase; 25.5, 44.3, 94.6 and 89.9 $\mu$g/mL respectively. The study showed, that after four weeks of treatment (aqueous *C. aconitifolius* extract) a hypoglycemic effect was observed in the animals, this can be related to insulin secretion or with inhibition of intestinal enzymes (amylase and glucosidase) and, suggests observing renal function in long-term intake of aqueous extract.

In table 5, it can be appreciated the different biological effects that several authors have reported in *C. aconitifolius* extracts. It has been observed that the aqueous and ethanolic extract have been the most used, presenting various effects such as; hepatoprotective, nephroprotective, hypoglycemic and antioxidant, but also the ethyl acetate and methanolic extract of *C. aconitifolius* have a protective effect. Few studies showed the effect of

administering *C. aconitifolius* leaves into the pellets and, have also exhibit protective effects (Oyagbemi et al., 2013).

Trough all studies presented above; the results suggest that *C. aconitifolius* can be an alternative treatment in LD an CKD. The use of chaya leaves or extracts may contribute significantly in protect the liver and the kidney before any damage occur, but also after hepatic and kidney injury (Adaramoye and Aluko, 2010; Akachukwu et al., 2014; Oyagbemi et al., (2013). It important to mention, then biological effects such as: antioxidant capacity, hypoglycemic and hypotensive effect, can also prevent NAFLD and CKD (Garcia-Rodriguez et al., 2014; Ramos-Gomez et al., 2016; Ajiboye et al., 2018).

**Table 5.** Summary of biological potential of *C. aconitifolius* extracts.

| **Extract** | **Biological potential** | | **Reference** |
|---|---|---|---|
| **Ethanolic** | Nephroprotection | Reduces $Na^+$, chlorine ion, creatinine and urea. | Orji *et al*., 2016 |
| | Hepatoprotection | Reduces blood levels of ALT, ALP, AST, LDL and BUN.<br>Increases albumin in blood. | Orji *et al*., 2016<br>Oyagbemi y Odetola, 2010 |
| | Hypoglycemic effect | Reduces glucose blood levels in diabetic mice. | Nath-Roy et al., 2016 |
| **Aqueous** | Protective | Stimulate the appetite, and there is no trace of toxicity in liver and kidney. | Adekomi *et al*., 2011 |
| | Hypoglycemic effect | Reduces the absorption of glucose at an intestinal level. | Ramos-Gomez *et al*., 2016 |
| | Hepatoprotection and nephroprotection | Histological activity without damage. | Akachukwu et al., 2014 |
| **Methanolic** | Endocrinology | Regulates the hormonal cycle. | Iyke *et al*., 2018 |
| | Nephroprotection by antioxidant capacity | Reduces the damage of the lypidic peroxidation. | Adaramoye and Aluko, 2010 |
| **Ethyl acetate** | Antioxidant | Binding of ions (ABTS). | Ajiboye et al., 2018 |
| | Hypoglycemic effect | Inhibition of α-amylase y α-glucosidase enzyme. | Ajiboye et al., 2018 |
| | Hypotensive effect | Inhibitory activity of angiotensive enzyme. | Ajiboye et al., 2018 |

## 7. Future prospects

CKD and LD are highly complex, as observed throughout the chapter. The treatment of both diseases requires various treatments; one with a nutritional approach and the other with drugs. At present, it is known that pharmacological treatment is expensive and has a lot of side effects, such as nausea, dizziness, diarrhea, vomiting, loss of appetite, etc. Medicinal plants like Chaya have been used over time to attend diseases. Several studies show the potential protective effect in the liver and kidney. Thus, chaya is a natural alternative, their possible side effects have to be studied, *in vivo* studies show no toxicity, and no side effects were observed. Therefore, it is expected that the different extracts of chaya can be used as pro-drugs for the treatment of CKD and LD, avoiding such side effects. One of the advantages of the extracts is that they are inexpensive, especially the aqueous extract, and the plant is highly available, since it is grown throughout all year long.

## CONCLUSIONS

Chaya (*C. aconitifolius*) is a food of great acceptance and low cost, in Mexico and different countries in the world. It has been used over the years in the gastronomy and natural medicine. Chaya offers a natural alternative, due to the biological effects that have been observed *in vitro* and *in vivo*. Allosteric inhibition by the secondary metabolites of the extracts, have an effect on intestinal enzymes, decrease the absorption of simple carbohydrates and as a result there is a decrease in blood glucose. This effect prevents the increase of insulin release, decreasing insulin resistance and at the same time prevents the production of free fatty acids in the liver, preventing the formation of fatty liver. The hypotensive effect is also observed in the extracts and can be attributed to the binding of the metabolites to the $Zn^{+2}$ site, preventing angiotensin I from becoming angiotensin II, and this is coupled to the AT I and II receptors, as well avoiding vasoconstriction at the systemic level. This effect is due to the phytochemicals that can be found in the plant and in the extracts of it. As mentioned, the phytochemicals can vary and depend on the solvent (polarity) used for the extraction. It can be concluded that chaya is a potential coadjuvant in the treatment of NCDs; such as CKD, LD, DM, insulin resistance and hypertension and avoid complications that can lead to dead.

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**ARTÍCULO DE INVESTIGACIÓN**

# "*In vitro* and *In vivo* hypoglycemic and hypotensive effect of six extracts of *Cnidoscolus aconitifolius* (Mill.) I.M. Johnst. leaves"

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**ABSTRACT:**
Non-Communicable Diseases (NCDs) such as diabetes and hypertension are the main cause of death in the world. Chaya (*Cnidoscolus aconitifolius* (Mill) I.M Johnst) is a shrub that is used for its medicinal properties in Mexico. The aim of this study was to evaluate hypotensive and hypoglycemic effect of six chaya leaves extracts (aqueous, ethanolic, acetone, ethyl acetate, diethyl ether and hexane). The extracts that had the lowest value of inhibitory concentration of 50 % ($IC_{50}$) for ACE, α-amylase and α-glucosidase were acetone (12.61 μg/mL), ethyl acetate (22.97 μg/mL) and ethyl acetate (3.20 μg/mL). Aqueous extract had the highest hypotensive effect by decreasing the systolic (15.3 %) and diastolic (23.4 %) blood pressure (BP) and, hexane extract had the highest hypoglycemic effect by decreasing glucose up to 22.88 % at 5.0 mg/kg dose. These results indicate that, all chaya extracts have hypotensive and hypoglycemic effect and could be used in the prevention of NCDs.

**Key Words:** ACE, α-amylase, α-glucosidase, *Cnidoscolus aconitifolius*, hypotensive effect, hypoglycemic effect.

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## 1. Introduction

One of the biggest challenges in the world is rapid rise of NCDs, since these are the main cause of mortality, with over 40 million people between the ages of 30 and 69 have died of it. NCDs, are also known as chronic diseases and are the result of genetic, physiological, environmental and behavioral factors (WHO, 2018). Principal types of NCDs are cardiovascular diseases, cancer, chronic respiratory diseases and diabetes mellitus (DM). There are factors than can increase the predisposition to develop a NCDs, such as excessive weight, hypertension, hyperglycemia and insulin resistance.

Nowadays, overweight and obesity play a key role in the development of hypertension and hyperglycemia. The hypertension can be defined as the increase in blood pressure above the normal parameters 120/80 mm/Hg (American Heart Association, 2017) being 130/80 and 140/90 mm/Hg considered as hypertension. The renin-angiotensin-aldosterone (RAA) cycle is an important aspect in the control of blood pressure; the overstimulation of this system leads to hypertension. In the RAA cycle, renin acts on the angiotensinogen that is released from the liver and hydrolyzes four amino acids (Val-Tyr-Ser-O) and angiotensin I is obtained. After that, the pulmonary endothelium releases the angiotensin-converting enzyme (ACE), which hydrolyzes two amino acids (His-Leu) and catalyzes the formation angiotensin II, this promotes the synthesis of aldosterone and the renal sodium reabsorption. The increase in angiotensin II concentrations, has as a consequence systemic vasoconstriction and increase in BP (Taler, 2018). In order, to avoid endothelial vasoconstriction, ACE inhibitors are prescribed such as captopril, this is the first option in the world for hypertension treatment. Captopril has proven to reduced morbidity and mortality in patients with hypertension. Nonetheless, ACE inhibitors have side effects such as diarrhea, nauseas, diminishes the intestinal microbiota, and they are contraindicated in liver disease and chronic kidney disease end stage (Méndez-Durán, 2011; Herrera-Chale et al., 2015).

Hyperglycemia is defined as the elevation of glucose in the blood stream and this could be due to the body has low or non-insulin production, or because insulin resistance in the organism (American Diabetes Association, 2018). Sustained hyperglycemia can lead to DM type II. Different medications are available to control blood glucose; there are three categories: biguanides (metformin), sulfonylureas (glibenclamide) and $\alpha$-glucosidase inhibitors (acarbose), each one has a different mechanism of action. Metformin acts on the liver and reduces glucose production and also decreases gluconeogenesis. Glibenclamide improves the body cell sensitivity to insulin and also stimulates PPAR-gamma receptor, these receptors are found mainly in fat cells. Acarbose is an inhibitor of the digestive absorptive process, it helps to slow down the absorption of carbohydrates, therefore the glucose absorbed by the bloodstream is lower and reduces the postprandial blood glucose (Linker and Humphreys., 2018). The medication for hyperglycemia like the hypotensive ones has side effects that affect the quality of life. Because of this, phytotheraphy have been proposed, to avoid side effects.

Chaya is a plant cultivated in the Mayan region of Guatemala, Belize, Mexico and Honduras. Of the four varieties of *C. aconitifolius*: estrella, picuda, chayamansa and redonda, only the chayamansa variety is edible (Ross-Ibarra & Molina-Cruz, 2006). In Mexico, the leaves of chaya are used since the pre-Hispanic ages, as an edible, medicinal and ornamental plant (Ross-Ibarra, 2004). In natural medicine, it was used to combat skin problems, eye problems, kidney stones and obesity (Akachukwu, Okafor, & Ibegbulem, 2014). The presence of protein and vitamins such as thiamine, niacin, riboflavin, retinol, beta carotene and ascorbic acid, and the presence of minerals such as: $Ca^{+2}$, $Fe^{+3}$, $PO_4^{-3}$, $K^{+}$, $Mg^{+2}$, $Na^{+}$, $Mn^{+2}$, $Zn^{+2}$ and $Cu^{+}$ and phytochemicals such as phenolic compounds, terpenes, alkaloids and sterols has been found in leaves and stems (Chikezie et al., 2016; Markus et al., 2016; Aye, 2012). *C. aconitifolius* it is also known for their cyanogenic glycosides; these are toxic due to cyanide molecule in it. The cyanogenic glycosides are removed with a heat process (≥ 40º C) that makes the hydrolysis of the hydrocyanic acid (Kuti & Konoru, 2006). Different studies suggest that biological activities of chaya leaves such as antioxidant, hypoglycemic and hypolypidic, are due to phytochemicals in the plant (Garcia-Rodrigez et al., 2014: Ramos-Gomez et al., 2016; Otitolaye and Asokan., 2016). In order to obtain these compounds an extraction process is required, the maceration involves soaking plant materials in a stoppered container with a solvent at a room temperature for a period of time (24, 48, 72 hours), with frequent agitation (Azwanida, 2015). This process intended to break down the plants cell wall and release the phytochemicals. Even thought, the family of phytochemicals will depend on different factors such as the solvent used, extraction method and time. The aqueous, ethanolic, methanolic and ethyl acetate extracts of chaya leaves had shown potential biological effects, such as antioxidant, hypoglycemic, hypotensive and hypolipidemic (Eze-Eme et al., 2017; García-Rodríguez et al., 2013; Ogunlade et al., 2009; Kuti & Konoru, 2004). This suggests that chaya leaves are potentially useful in the treatment of hyperglycemia and hypertension.

The aim of this study was to evaluate hypotensive and hypoglycemic effect of six extracts obtained by maceration of *C. aconitifolius* leaves in different studies.

## 2. Material and methods

### 2.1 Plant material and chemicals

Leaves of *Cnidoscolus aconitifolius* (Mill) I.M Johnst were collected in Timucuy, Yucatan, Mexico. A voucher specimen of the plant was deposited at Center of Scientific Investigation in Yucatan (CICY) given the #69489. Afterwards, leaves were wash and disinfect, dry in a conventional oven at a 40 ºC temperature for 24 hours (Otitolaiye & Asokan, 2016). Then, the dry leaves were grounded in a Cyclotec mill (Tecato, Höganas, Sweden) to obtain a chaya flour that was preserved in a plastic bag inside a desiccator (Akachukwu et al., 2014). All chemicals were reagent grade or better and purchased from Sigma Chemical Co.

### 2.2 Extraction yield

Maceration for 48 h was the technique selected for the extraction process, with a proportion of 1:10 (w/v). Six solvents were selected: distilled water, ethanol, acetone, ethyl acetate, diethyl ether and hexane. After the time passed, extracts were filtered using No. 50 filter paper and concentrated under reduced pressure. At last, the extracts were put in the rotary vacuum evaporator, then frozen, lyophilized and store at 4 ºC until further analysis (Akachukwu et al., 2014; Otitolaiye & Asokan, 2016).

### 2.3 Biological activities

#### 2.3.1 ACE inhibitory

The ACE inhibitory activity was determined according to Hayakari et al., (1978), in which the ACE hydrolyzed Hipuril-L-Histidil-L-Leucine (HHL) to hipuric acid and His - Leu. This method is based on the colorimetric reaction of hippuric acid with TT (2,4,6-trichloro-triasin). First, 20 µL of ECA solution (100 mU / mL) and 40 µL of chaya extract were added and incubated at 37 °C for 5 min. Then, 100 µL of HHL (hipuryl histidyl leucine) prepared at a concentration of 0.3% in a mixture of 40 µmol potassium phosphate buffer and 300 µmol sodium chloride buffer previously adjusted to pH 8.3 with HCl or NaOH were added, and incubated at 37 °C for 45 min. The reaction was stopped by adding of 360 µL of TT solution in dioxane (3% w/v) and 720 µL phosphate buffer 0.2 M (pH 8.3). Finally, it was centrifuged at 10,000 revolutions per minute (rpm) for 10 min.

The supernatant was obtained and read at an absorbance of 382 nm. These tests were performed in triplicates.

The percentage of ACE inhibitory activity was determined with the following equations:

$$\% \text{ ACE inhibitory} = (A - B) / (A - C) \times 100$$

Where A represents the absorbance in the presence of the sample with ACE, B the absorbance of the control and C the absorbance of the target reaction.

The concentration of the extract necessary to inhibit the activity of the ACE in 50% ($IC_{50}$) was expressed in: $Y = a + b * \ln(x)$.

Where:
Y = inhibition percentage
a = is the intersection
b = is the slope
x = concentration

### 2.3.2 α-glucosidase and α-amylase inhibition assay

The inhibitory activity of the α-glucosidase enzyme was analyzed according Dineshkumar et al., (2010). In which, the α-glucosidase (2 U/mL) was mixed with 20 µL of chaya extract at different concentrations (10, 20, 30, 40 and 50 µg/mL). After that, the enzyme and the extract were incubated for 5 min at 37 °C. Then, 20 µL of p-NPG (p-nitrophenyl glucopyranoside) was added at 1 mM with a phosphate buffer (50 mM) at pH 6.8, the above in order to start the reaction. This mixture was incubated for 30 min at 37 °C and the reaction was stopped by adding 50 µL of $Na_2CO_3$ (sodium carbonate) to 1 M, to a final volume of 150 µL. Finally, the activity of α-glucosidase was read at an absorbance of 405 nm. This process was done in triplicates.

The inhibitory activity of the α-amylase enzyme was analyzed also by Dineshkumar et al., (2010). In which, 2 mg of starch was added in a tube with 0.2 mL of Tris-HCL buffer at 50 mM (pH 6.9) with calcium chloride at 0.01 M. The test tubes were heated for 5 min at 60 °C, then incubated at 37 °C for 5 min. Chaya extracts (100 µg/mL) were dissolved in DMSO at 50 % to obtain five concentrations (20, 40, 60, 80 and 100 µg/mL). Then, 0.2 mL of the extract of the corresponding concentration was added. Next, 0.1 mL of porcine pancreatic amylase (2 U/mL) was added to the Tris-HCl buffer in the test tubes containing starch (positive control) and extracts. This process was incubated at 37 °C for 10 min. The reaction was stopped by adding 0.5 mL of 50% acetic acid and read at an absorbance of 540 nm. This test was performed in triplicates.

Percent of inhibition of the activity of α-glucosidase and α-amylase was determined with the following equations:

Inhibition of α-glucosidase and α-amylase activity:

$$\text{Inhibition (\%)} = ((Ac^{+}) - (Ac^{-}) - (As - Ab)/(Ac^{+}) - (Ac^{-})) \text{ X } 100$$

Where $\mathbf{Ac^{+}}$ is the absorbance when the enzyme acts without interference (solvent with enzyme), $\mathbf{Ac^{-}}$ is the absorbance when the enzyme does not act (solvent without enzyme), **As** is the absorbance when the enzyme acts in the presence of sample (sample with enzyme) , **Ab** is the absorbance of the blank (sample without enzyme).

The concentration of the extract necessary to inhibit the activity of the α-glucosidase and α-amylase in 50% ($IC_{50}$) was expressed in: $Y = a + b * \ln(x)$.

Where:
Y = inhibition percentage
a = is the intersection
b = is the slope
x = concentration

### 2.3.3 *In vivo* experimentations

#### 2.3.3.1 Animals

Male Wistar rats (n = 40) were used (8 -12 weeks), with a starting weight of 247.6 ± 38.4 g. The animals were obtained from the bioterium of the Faculty of Medicine of the Autonomous University of Morelos; were household at room temperature of 25 ºC, with 12/12 h light-dark cycle. Animals were divided in eight groups of five rats: six groups for treatments, one for positive control and the another one for negative control. Experiments were performed in accordance with the Mexican Official Law Guidelines for Animal Testing (NOM-062-Z00-1999).

#### 2.3.3.2 Obesity Induction

Animals were induced to obesity, hyperglycemia and hypertension by administering water with 20 % of sucrose *ad libitum*, this treatment was provided for twelve weeks in order to increase the weight of the rats. After this period of time, animals were measure according to Lee et al., (1996) to confirm obesity.

#### 2.3.3.3 Hypotensive effect

Blood pressure (BP) was determined according the modified methodology of Herrera-Chale et al., (2014). In where the six chaya extracts; aqueous, ethanol, acetone, ethyl acetate, diethyl ether and hexane were evaluated, at a single dose of 5 mg/kg, these by injecting the extracts intraperitoneally in a volume of 100 μL per rat. As a positive control, captopril (Sigma C4042) was used at the same dose. All rats had free access to a standard food (nu3lab, Research Global Solution) and water with 20% of sucrose. The BP was determined with an sphygmomanometer for rat's model Kent Scientific CODA Non-invasive standard for pressure, acquired from Scientific Senna, the experiment was performed for at least 2.5 h per group, the first 20-30 min of the experiment represented basal BP and then the treatment was administered intraperitoneally and monitored for 30, 60 and 120 min.

#### 2.3.3.4 Glucose tolerance curve (GTC)

GTC was determined according to Parra-Naranjo et al., (2017). Five rats per treatment were used, and all chaya extracts were evaluated at two doses (0.5 and 5 mg/kg). The extracts were administered in conjunction with 1 g/kg starch dissolved in distilled water, for positive control acarbose was used. The administration was carried out orally with stainless steel cannulas for feeding rats. Previously the Wistar rats were fasted for 8 h, and during the experiment (120 min) they had no access nor water or food. The glucose blood levels were determined with a micro-drop of blood taken from the tip of the tail with a portable analyzer (Accu-chek® Performa), six measurements were taken at 0, 15, 30, 45, 60 and 120 min. The results obtained were normalized by dividing each data, between the basal content and a certain time.

#### 2.3.3.5 Hypoglycemic effect

Hypoglycemic effect was determined according Parra-Naranjo et al., (2017); five rats per treatment with no previous fasting were used. Then, all chaya extracts were evaluated at

two doses (1 and 5 mg/kg) by dissolving them in distilled water, for positive control glibenclamide was used. Administration of the extracts was performed intraperitoneally by using 1 mL sterile insulin syringes. Once the experiment was started, the animals were deprived of food and water, and the blood levels were determined with a micro-drop of blood taken from the tip of the tail with Accu-chek® Performa a portable analyzer, seven measurements were taken at 0, 1, 2, 3, 4, 5, and 6 h. The results obtained, were normalized by dividing each data, between the basal content and a certain time.

### 2.4 Statistical analysis

All results were analyzed using central tendency and dispersion measures. One-way ANOVA was run to evaluate the extraction yield data, the $IC_{50}$ of ACE, $\alpha$-glucosidase, $\alpha$-amylase enzymes, the hypotensive effect, glucose tolerance curve and hypoglycemic effect data. After that, a Tukey test was performed to determine differences between treatments and Dunnett test to determine difference between treatments and control, $p<0.05$. Also, $IC_{50}$ was determined using non-linear regression analysis. All analyses were done according to Montgomery, (2007) and processed with Statgraphics Centurion version XVII.II and Graphpad Prism 6.0 software.

## 3. Results and discussion

### 3.1 Extracts yield

In the present study, chaya extracts exhibited a yield between 6.99% ± 0.27 to 25.68% ± 0.62 of dry weight (Figure 1), this is similar to what was reported for Otitolaiye & Asokan, (2015), with a proportion of 1:10. It can be observed that polar solvents had a higher percentage of yield, such as aqueous extract and ethanol, compared with non-polar extracts such as hexane that had lower yield.

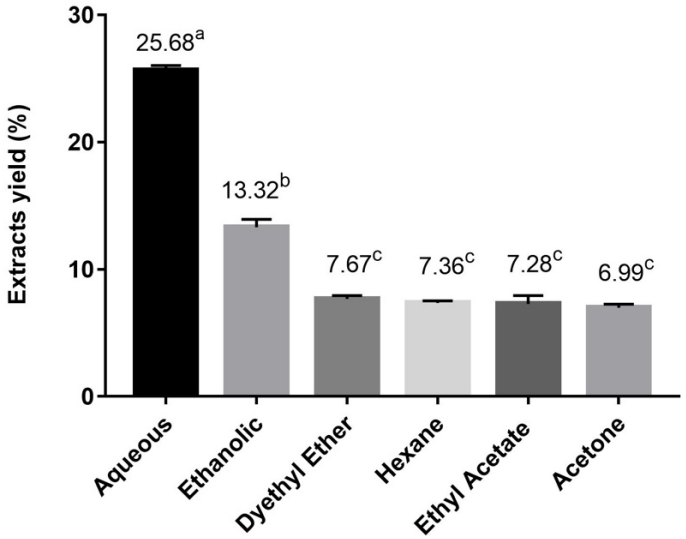


**Figure 1.** Yield of six extracts of *C. aconitifolius*, aqueous, ethanolic, diethyl ether, hexane, ethyl acetate and acetone by 48 h maceration process. [a-c]Different superscript letters indicate statistical difference between extracts by One-way ANOVA and Tukey´s multiple range test. Data expressed as means ± SD, n = 3, ($p<0.05$).

The main reason to select different dissolvent, is to obtain a variety of phytochemicals. The polarity index is important due to its relationship to the structure of phytochemicals; polar solvents have mayor affinity to phenolic compounds and non-polar solvents have affinity to terpenoids and sterols. It is known than each family of phytochemicals can have a specific biological activity. Studies suggests that phenolic compound have antioxidant effect in chronic diseases and terpenoid and sterols have and hypoglycemic effect (Akachukwu et al., 2014; Altemimi, Lakhssassi, Baharlouei, Watson, & Lightfoot, 2017).

### 3.2 ACE, α-amylase and α-glucosidase inhibitors

All six chaya extracts exhibited inhibition on ACE and α-amylase and α-glucosidase enzymes. The $IC_{50}$ of ACE, α-amylase and α-glucosidase activity was expressed in μg/mL and, smaller values of $IC_{50}$ indicated greater inhibition activity. A positive control was used in each assay, for the ACE inhibitory activity Captopril was used with an $IC_{50}$ 11.58 μg/mL and for the α-amylase and α-glucosidase activity Acarbose with an $IC_{50}$ 33.89 μg/mL.

#### 3.2.1 ACE inhibitory activity

In the present study, all chaya extracts exhibit ACE inhibitory activity and have $IC_{50}$ between 12.61 and 103.46 μg/mL. The results showed that acetone extract had the lowest $IC_{50}$ (12.61 ± 0.07 μg/mL) and had statistical difference ($p < 0.05$) between the other extracts, also is the only extract that had no statistical difference ($p > 0.05$) respect the positive control (Table 1). It has been show that acetone extracts had the presence of phenolic compounds such as flavonoids, phenols, coumarin and tannins, these is similar what is extracted in the ethanol extract (Altemimi et al., 2017; García-Rodríguez et al., 2014). Diverse studies exhibited the activity of flavonoids to inhibit ACE (Chua, 2013; Guerrero et al., 2012; Larson, Symons, & Jalili, 2012; Madaka, Pathompak, Sakunpak, Monton, & Charoonratana, 2017). Quercetin, rutin and kaempferol had been reported in the *C. aconitifolius* extracts (Otitolaiye & Asokan, 2016). According to Guerrero et al. (2012) the structure of the flavonoids is important to inhibit the ACE enzyme; characteristics such as the double bond between C2 and C3 at the C-ring, or the cetone group at the C4 carbon on the C-ring are essential for this inhibition. Quercetin and rutin had the presence of unsaturated 2-3 bond that is conjugated with a 4-oxo function besides the 3´4´-cathechol ring that also inhibit the ACE site. These phytochemicals are present in the acetone extract of *C. aconitifolius* (Guerrero et al., 2012; Larson et al., 2012). Furthermore, some studies suggest than antioxidant capacity of flavonoids, is useful antagonizing vasoconstriction, when stimulating the production of nitric oxide a potent vasodilator (Guerrero et al., 2012).

**Table 1**. $IC_{50}$ of ACE, α-amylase and α-glucosidase inhibition of the six extracts of *C. aconitifolius.*

| Extract | $IC_{50}$ (μg/mL) | | |
|---|---|---|---|
| | **ACE** | **α-amylase** | **α-glucosidase** |
| **Aqueous** | $65.73 \pm 0.55^{d,B}$ | $47.04 \pm 6.44^{b,A}$ | $8.97 \pm 0.96^{b,B}$ |
| **Ethanolic** | $82.36 \pm 1.29^{e,B}$ | $43.08 \pm 19.02^{ab,A}$ | $5.08 \pm 2.23^{a,B}$ |
| **Diethyl ether** | $23.29 \pm 3.86^{b,B}$ | $34.12 \pm 8.23^{ab,A}$ | $9.77 \pm 1.80^{b,B}$ |
| **Hexane** | $39.76 \pm 2.39^{c,B}$ | $36.26 \pm 1.68^{ab,A}$ | $16.59 \pm 1.14^{c,B}$ |
| **Ethyl acetate** | $103.46 \pm 2.11^{f,B}$ | $22.97 \pm 1.11^{a,A}$ | $3.20 \pm 0.54^{a,B}$ |
| **Acetone** | $12.61 \pm 0.07^{a,A}$ | $88.27 \pm 2.01^{c,B}$ | $4.49 \pm 0.66^{a,B}$ |

[a-f]Different superscript letters in the same column indicate statistical difference between extracts by One-way ANOVA and Tukey´s multiple range test. [A-B]Different capitals letters in the same column indicate statistical difference between treatment and positive control by One-way ANOVA and Dunnett test. Data expressed as means ± SD, n = 3, ($p<0.05$).

### 3.2.2 α-amylase and α-glucosidase inhibition assay

The results showed that all extracts exhibited α-glucosidase and α-amylase inhibition and $IC_{50}$ range between 3.20 – 16.59 and 22.97 – 88.27 μg/mL, respectively. Ethyl acetate extract of chaya leaves had the lowest $IC_{50}$ (22.97 ± 1.11 μg/mL) to inhibit α-amylase enzyme, but also the diethyl ether, hexane and ethanolic extract had low concentrations of $IC_{50}$ and had no statistical difference ($p > 0.05$) between them; the acetone extract is the only one that had significant difference ($p < 0.05$) with the control acarbose (Table 1). As well, the ethyl acetate extract of chaya had the lowest $IC_{50}$ (3.20 ± 0.54 μg/mL) to inhibit α-glucosidase enzyme, and had no statistical difference ($p > 0.05$) with the acetone and ethanolic extract, all of the *C. aconitifolius* extract had statistical difference with the control (acarbose), due to the control (acarbose) $IC_{50}$ 33.89 μg/mL is higher than reported with chaya extracts.

The phytochemicals from the ethyl acetate extract of *C. aconitifolius* have been reported with high-perfomance liquid chromatography (HPLC) and, it showed the presence of coumaric acid, amentoflavone, dihydromyricetin, protocatechuic acid, kaempferol, hesperidin, quercetin and rutin, have been reported to show hypotensive activity (Ajiboye, Ojo, Okesola, Oyinloye, & Kappo, 2018; Guerrero et al., 2012). Meanwhile, the structure-activity relationship of flavonoids it is important toward to inhibit the α-amylase and α-glucosidase enzymes (Juárez-Reyes, Escandón-Rivera, Mata, Rivero-Cruz, & Cristians, 2013; Martinez-Gonzalez, Díaz-Sánchez, de la Rosa, Bustos-Jaimes, & Alvarez-Parrilla, 2019; Proença et al., 2017). Specially, hesperidin and quercetin have the capacity to inhibit the α-amylase enzyme by making hydrogen bonds and hydrophobic bindings. Additionally, the double bond between C2 and C3 A-ring, the catechol structure in B-ring

and planarity of C-ring are characteristics that a flavonoid must have to inhibit this enzyme (Martinez-Gonzalez et al., 2019). It has been studied the ability to inhibit the α-glucosidase by flavone, kaempferol, quercetin and rutin. Studies suggest that quercetin showed a competitive inhibition in the active site of the enzyme by making two hydrogen bonds in the active site. It is known, that -OH groups in the B-ring are favorable to inhibit the α-glucosidase enzyme (Juárez-Reyes et al., 2013; Proença et al., 2017) this type of flavonoids have been found in the ethyl acetate, aqueous, ethanolic extract of *C. aconitifolius,* this suggest the ability of the chaya extracts to inhibit intestinal enzymes (Ajiboye et al., 2018; Aye, 2012; Otitolaiye & Asokan, 2016; Ramos-Gomez et al., 2016). The $IC_{50}$ value for α-glucosidase of the ethyl acetate extract of *C. aconitifolius* was 3.20 μg/mL. This value is lower than reported by Ajiboye et al., (2018) for ethyl acetate extract of C. aconitifolius (18.98 μg/mL) and, the $IC_{50}$ value for α-glucosidase and α-amylase inhibition for the aqueous extract was 47.04 and 8.97 μg/mL respectively. These values are lower than reported by Ramos-Gomez et al., (2016) for aqueous extract of *C. aconitifolius* leaves (94.6 and 89.9 μg/mL respectively).

These results suggest that the inhibition effect is caused by the phenolic compounds (flavonoids) such as catechin and chlorogenic acid that inhibit α-amylase enzyme (Ramos-Gomez et al., 2016). Furthermore, the cardiac glycosides, such as quercetin 3-glucoside and kaempferol have shown inhibition in the active transport of glucose in the ileum (Jiménez-Arellanes, García-Martínez, & Rojas-Tomé, 2014). It is well know that inhibition of the intestinal enzymes decreases the glucose release from starch, this will result in the delay of glucose absorption through the intestinal lumen (Schnell et al., 2016).

### 3.3 Rat model with obesity hyperglycemia and hypertension

After twelve weeks of water with sucrose (20 %) intake, the animals gain 314.2 g approximately. However, the Lee et al., (1996) was taken to confirm obesity of the rats by weighing them and measuring the distance between snout and tale. After that, the result was a 0.309 index, this score means obese rats.

#### 3.3.1 Hypotensive effect in obese rats

ACE inhibitory activity not always correlate with the *in vivo* hypotensive effect. In order to observe the hypotensive effect *in vivo*, the six extracts of chaya were injected intraperitoneally to male hypertensive Wistar rats in a single dose (5 mg/kg). After that, the systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measure. Hypertensive Wistar rats presented a mean of SBP/DBP of 126.8/103.3 mmHg, the variations in SBP and DBP with treatment and Captopril are shown in Table 2. The administration of aqueous extract of chaya decreased DBP up to 23.4 %, this value is higher than the exhibit by Captopril (19.4 %) at 30 min (Figure 2). The highest decreased in SBP was observed with the aqueous (15.3 %) and ethanolic (15.3 %) extract and had no statistical difference with Captopril ($p > 0.05$). Overall, the maximal decreasing of SBP and DBP was observed with the aqueous extract from 129.6 to 86.3 mmHg and 108.4 to 74 mmHg, respectively and had statistical difference with Captopril ($p < 0.05$). The ethyl

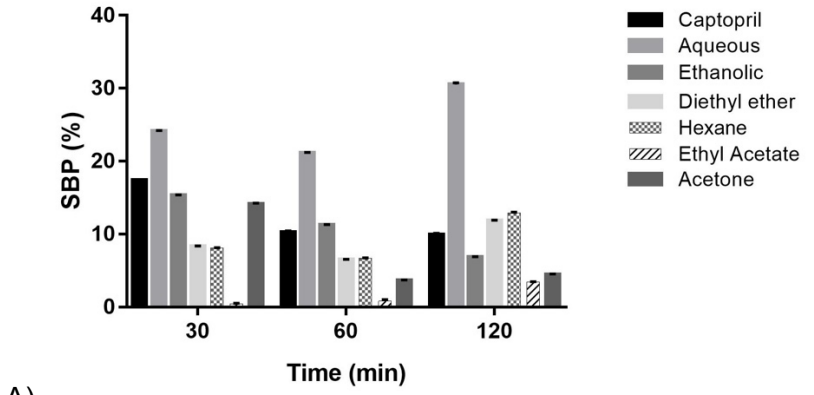
acetate extract was the only one that did not decrease the SBP at 30 min. Meanwhile, the aqueous, hexane and diethyl ether extracts of chaya they continue decreasing the SBP and DBP during all the experiment. This behavior is important, due to hypertensive medications could lose its effectiveness after 30 minutes. The aqueous extract of *C. aconitifolius* was found to possess hypotensive effect by decreasing the SBP and DBP after 2 h. Phenolic compounds have been correlated to a hypotensive effect.

Another studied exhibit the presence of borneol a monoterpene in aqueous extract; this compound has been isolated and have also shown hypotensive effect (Mordi et al., ;Otitolaiye & Asokan, 2016). Flavonoid compound rutin have shown antioxidant effects with 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric-reducing antioxidant power (FRAP) and phosphomolobdenum-reducing antioxidant power (PRAP), suggesting the capacity to prevent the oxidation from reactive oxygen species (ROS) like nitric oxide ($NO^-$), which is a potent vasodilator that prevent the side effects of angiotensin II elevation (Chua, 2013; Taler, 2018). Another mechanism involved in hypertension is the renin-angiotensin-aldosterone cycle (Kalantar-Zadeh & Fouque, 2017; Taler, 2018). Docking studies have exhibit the structure-relationship with flavonoids (quercetin, rutin and kaempferol) that can inhibit ACE action site, resulting in a hypotensive effect (Ajiboye et al., 2018; Guerrero et al., 2012). According to Emelike and Unegbu (2015), the hypotensive effect of leaves of *C. aconitifolius*, in slightly hypertensive rats, showed the SBP decreased up 128.8 to 126.2 mmHg, representing a decrease of 2.01%, and the DBP did not decrease, this only by given the leaves directly to the rats, these results suggest that the extracts of *C. aconitifolius* have greater effect than only the leaves (Altemimi et al., 2017; Oyagbemi & Odetola, 2013).

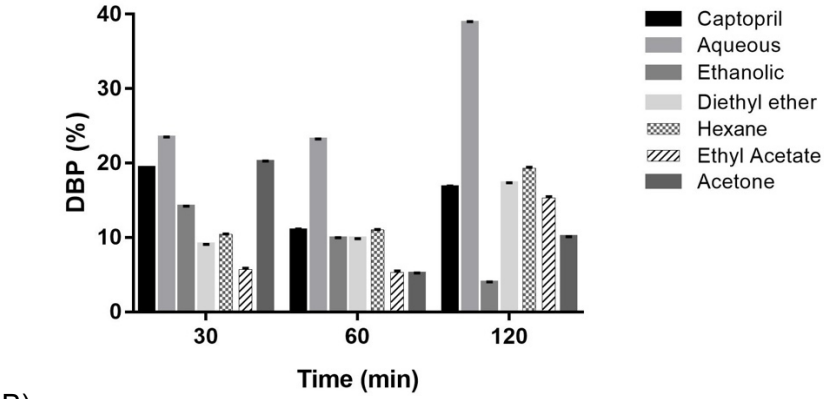
**Table 2.** Results of SBP and DBP (mm/Hg) on rats after the administration of the extracts of *C. aconitifolius*.

| Extract (5 mg/kg) | Time (min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 30 | | 60 | | 120 | |
| | SBP | DBP | SBP | DBP | SBP | DBP | SBP | DBP |
| **Aqueous** | 129.7 ± 17.7 | 108.3 ± 16.2 | 108.3 ± 10.1$^{a,A}$ | 81.7 ± 10.6$^{a,A}$ | 106.3 ± 81.6$^{a,A}$ | 81.6 ± 5.9$^{a,B}$ | 98.3 ± 16.4$^{a,B}$ | 74.0 ± 11.1$^{a,A}$ |
| **Ethanolic** | 135.7 ±16.9 | 109.3 ± 18.0 | 113.7 ± 1.9$^{a,A}$ | 92.1 ± 4.6$^{ab,A}$ | 119.2 ± 2.4$^{ab,A}$ | 96.2 ± 1.0$^{ab,A}$ | 125.4 ± 11.0$^{ab,A}$ | 103.4 ± 9.1$^{b,B}$ |
| **Diethyl ether** | 137.7 ± 16.7 | 112.4 ±13.4 | 126.1 ± 16.1$^{ab,A}$ | 101.9 ± 13.0$^{ab,A}$ | 128.8 ± 17.8$^{ab,A}$ | 101.3 ± 12.9$^{ab,A}$ | 121.6 ± 22.6$^{ab,A}$ | 93.2 ± 92.4$^{ab,A}$ |
| **Hexane** | 138.7 ± 13.4 | 114.1 ± 10.3 | 127.4 ± 14.4$^{ab,A}$ | 102.1 ± 11.9$^{ab,A}$ | 129.6 ± 17.0$^{ab,A}$ | 101.6 ± 10.6$^{ab,A}$ | 121.1 ± 23.2$^{ab,A}$ | 92.4 ± 20.7$^{ab,A}$ |
| **Ethyl acetate** | 123.8 ± 9.7 | 105.2 ± 11.8 | 123.5 ± 18.1$^{b,B}$ | 98.9 ± 20.1$^{b,A}$ | 122.8 ± 27.4$^{b,B}$ | 99.3 ± 24.4$^{ab,A}$ | 119.8 ± 16.1$^{ab,A}$ | 88.7 ± 19.0$^{ab,A}$ |
| **Acetone** | 138.7 ± 15.2 | 113.9 ± 10.8 | 125.4 ± 24.4$^{ab,A}$ | 97.8 ± 21.2$^{ab,A}$ | 136.0 ± 15.7$^{ab,A}$ | 113.8 ± 17.1$^{b,A}$ | 136.3 ± 19.6$^{b,A}$ | 107.4 ± 16.9$^{b,B}$ |
| **Captopril®** | 126.6 ± 13.5 | 103.5 ± 12.4 | 103.5 ± 4.2$^{A}$ | 82.1 ± 5.1$^{A}$ | 111.0 ± 16.4 | 90.1 ± 13.9$^{A}$ | 112.7 ± 19.0$^{A}$ | 85.2 ± 17.0$^{A}$ |

$^{a-b}$Different superscript letters in the same column indicate statistical difference between extracts by One-way ANOVA and Tukey´s multiple range test.
$^{A-B}$Different capitals letters in the same column indicate statistical difference between treatment and positive control by One-way ANOVA and Dunnett test. Data expressed as means ± SD, n = 5, ($p<0.05$).




A)




B)

**Figure 2.** Percentage of BP reduction after the administration of six extracts of *C. aconitifolius*, aqueous, ethanolic, diethyl ether, hexane, ethyl acetate, acetone and Captopril, A) SBP and B) DBP. Data expressed as means ± SD, n = 5.

### 3.3.2 GTC in obese rats

One important mechanism in hyperglycemia is absorption via intestinal enzymes. Through the inhibition of intestinal enzymes ($\alpha$-amylase and $\alpha$-glucosidase), it can be delay the glucose absorption and released to the bloodstream (Dineshkumar, Mitra, & Mahadevappa, 2010). Overall in this study, the *in vitro* tests showed that all chaya extracts had inhibition of this enzymes; in order to explore this effect *in vivo*, male Wistar rats were induced with hyperglycemia and obesity allowing to evaluate abnormalities in the glucose metabolism. Table 3 shows the GTC results at two different doses (0.5 and 1.0 mg/kg). As mention previously, positive control (acarbose) was used in both doses. At the 0.5 mg/kg dose, basal blood glucose levels were between 102.6 – 116.0 mg/dL, during the first 15 min it was observed that all chaya extract raised up glucose blood levels (121.6 – 137.2 mg/dL), this is expected due to the administration of starch. At 30 min the ethanolic and hexane extract started to decrease the glucose in 4.2 and 5.1 %, respectively. The results showed that, hexane extract had a higher percentage of glucose inhibition, reducing glucose levels from 116 to 107.2 mg/dL, having a greater effect than acarbose 109.6 to 108.8 mg/dL. Meanwhile, the extracts: ethyl acetate, diethyl ether, ethanol and acetone had the same percentage of glucose reduction and had no statistical difference ($p > 0.05$).

At the 5.0 mg/kg dose of chaya extracts, basal blood glucose was between 103.4 – 109.6 mg/dL, 15 minutes after the administration of starch with chaya extracts, glucose levels raised up (110.4 – 135.8 mg/dL). After 30 min have passed, the acetone extract decreased glucose from 125.2 to 119.2 mg/dl, while the other chaya extracts exhibit hypoglycemic effect until 60 min have passed. Finally, at 120 min glucose blood levels were similar in all chaya extracts (103.4 – 109.4 mg/dL) and, these values were lower than observed with acarbose (110.8 mg/dL). Overall the chaya extracts decreased the glucose between 12.7 – 23.0 %, these results are similar to those obtained by Ramos-Gomez et al., (2016), who reported that aqueous extract of *C. aconitifolius* can decreased blood glucose by approximately 18 to 25 % in diabetic rats.

The results found in this study suggest that acetone and hexane extract of *C. aconitifolius* have a greater effect by reducing glucose after the intake of starch (García-Rodríguez et al., 2014). Not only phenolic compounds are able to inhibit $\alpha$-amylase and $\alpha$-glucosidase, also terpenes and sterols have the capacity to inhibit these enzymes. According to Garcia-Rodriguez et al., (2014) the hexane extract of *C. aconitifolius*, have the presence of lupeol, $\alpha$-amirina, $\beta$-amirina and cardiotonic glycoside. These compounds have shown inhibition on intestinal enzymes (amylase and glucoside). Meanwhile, pentacyclic triterpenes (ursane and oleane type) from hexane extract can inhibit the intestinal enzymes uncompetitively by forming hydrogen bonds with an important amino acid surrounding the catalytic site of the enzyme and also by inhibit the glycosylation end products of the glycolysis (Juárez-Reyes et al., 2013; Nazaruk & Borzym-Kluczyk, 2015). Few studies of $\alpha$-amirina and $\beta$-amirina, have exhibit lowering glucose in blood by inhibit the $\alpha$-amylase and $\alpha$-glucosidase (Furtado et al., 2017).

**Table 3.** Results of the glucose (mg/dL) in the GTC of six extracts of *C. aconitifolius* at two doses 0.5 and 5.0 mg/kg for 120 minutes.

| **Extract** | **Time (min)** | | | | | |
|---|---|---|---|---|---|---|
| **(**0.5 mg/kg**)** | **0** | **15** | **30** | **45** | **60** | **120** |
| **Aqueous** | 110.2 ± 3.1 | 137.2 ± 18.9$^{a,A}$ | 147.8 ± 27.2$^{b,a}$ | 139.6 ± 25.9$^{a,A}$ | 142.4 ± 31.0$^{b,A}$ | 117.6 ± 10.4$^{b,A}$ |
| **Ethanolic** | 109.2 ± 4.2 | 137.2 ± 9.2$^{a,A}$ | 131.4 ± 8.0$^{ab,A}$ | 126.0 ± 10.9$^{a,A}$ | 123.8 ± 4.5$^{ab,A}$ | 108.4 ± 6.6$^{ab,A}$ |
| **Diethyl ether** | 102.6 ± 8.3 | 121.6 ± 8.3$^{a,A}$ | 123.0 ± 16.6$^{ab,A}$ | 118.0 ± 9.4$^{a,A}$ | 124.6 ± 8.7$^{ab,A}$ | 106.2 ± 6.7$^{ab,A}$ |
| **Hexane** | 116.0 ± 15.7 | 133.4 ± 7.4$^{a,A}$ | 126.6 ± 5.6$^{a,A}$ | 125.6 ± 3.2$^{a,A}$ | 125.2 ± 9.1$^{a,A}$ | 107.2 ± 9.9$^{a,A}$ |
| **Ethyl acetate** | 108.8 ± 5.9 | 134.8 ± 13.1$^{a,A}$ | 133.2 ± 6.4$^{ab,A}$ | 137.8 ± 6.1$^{a,A}$ | 129.6 ± 3.8$^{ab,A}$ | 108.8 ± 4.1$^{ab,A}$ |
| **Acetone** | 105.8 ± 4.6 | 130.0 ± 8.7$^{a,A}$ | 129.6 ± 8.4$^{ab,A}$ | 122.4 ± 11.6$^{a,A}$ | 119.2 ± 8.2$^{ab,A}$ | 109.2 ± 6.6$^{ab,A}$ |
| **Acarbose®** | 109.6 ± 9.8 | 144.8 ± 30.1$^{A}$ | 140.8 ± 23.3$^{A}$ | 136.4 ± 14.5$^{5}$ | 131.8 ± 19.4$^{A}$ | 108.8 ± 10.4$^{A}$ |
| **Extract** | **Time (min)** | | | | | |
| **(**5.0 mg/kg**)** | **0** | **15** | **30** | **45** | **60** | **120** |
| **Aqueous** | 106.2 ± 3.9 | 124.0 ± 12.3$^{abc,B}$ | 124.6 ± 12.9$^{a,A}$ | 124.6 ± 7.5$^{a,A}$ | 122.4 ± 8.1$^{ab,A}$ | 108.8 ± 7.8$^{a,A}$ |
| **Ethanolic** | 110.2 ± 6.2 | 110.4 ± 5.9$^{a,B}$ | 119.6 ± 7.8$^{a,B}$ | 122.0 ± 5.8$^{a,B}$ | 119.4 ± 2.9$^{a,A}$ | 107.0 ± 8.3$^{a,A}$ |
| **Diethyl ether** | 103.4 ± 5.8 | 117.2 ± 5.8$^{ab,B}$ | 118.0 ± 8.2$^{a,A}$ | 120.8 ± 11.3$^{a,A}$ | 119.0 ± 11.0$^{ab,A}$ | 103.4 ± 4.8$^{a,A}$ |
| **Hexane** | 109.6 ± 5.5 | 126.6 ± 9.7$^{ab,B}$ | 129.2 ± 8.8$^{a,A}$ | 122.6 ± 4.2$^{a,A}$ | 121.4 ± 7.9$^{ab,A}$ | 109.4 ± 4.4$^{a,A}$ |
| **Ethyl acetate** | 106.0 ± 6.9 | 135.8 ± 9.9$^{bc,A}$ | 145.0 ± 10.1$^{b,A}$ | 129.2 ± 8.8$^{a,A}$ | 132.6 ± 11.1$^{b,A}$ | 104.6 ± 9.8$^{a,A}$ |
| **Acetone** | 104.4 ± 4.9 | 125.2 ± 6.4$^{bc,A}$ | 119.2 ± 10.1$^{a,A}$ | 117.6 ± 13.3$^{a,A}$ | 116.2 ± 15.5$^{a,B}$ | 106.4 ± 5.2$^{a,A}$ |
| **Acarbose®** | 111.6 ± 9.8 | 140.8 ± 30.1$^{A}$ | 138.8 ± 23.3$^{A}$ | 136.4 ± 14.5$^{A}$ | 131.8 ± 19.4$^{A}$ | 110.8 ± 10.4$^{A}$ |

$^{a-c}$Different superscript letters in the same column indicate statistical difference between extracts by One-way ANOVA and Tukey´s multiple range test. $^{A-B}$Different capitals letters in the same column indicate statistical difference between treatment and positive control by One-way ANOVA and Dunnett test. Data expressed as means ± SD, n = 5, ($p<0.05$).

### 3.3 Hypoglycemic effect in obese rats

Another mechanism to evaluate chaya extracts in the glucose metabolism is the hypoglycemic effect (Parra-Naranjo et al., 2017). In this experiment animals were not fasted. Afterwards, basal glycemia was obtained for the treatments (chaya extracts) and control (Glibenclamide) intraperitoneally. Table 4 shows the hypoglycemic effect of the chaya extracts with a dose of 1.0 mg/kg. Within the first hour, the glucose decreased with the diethyl ether 122.4 to 119.6 mg/dL (2.29 %), acetone 121.8 to 117.2 mg/dL (3.77 %), aqueous 115.4 to 108.4 mg/dL (6.06 %), ethyl acetate 122.6 to 114.0 mg/dL (7.01 %) and hexane 126.4 to 115.4 mg/dL (8.7 %) extracts. Meanwhile the ethanolic extract of chaya showed effect until the third hour 113.8 to 111.6 (1.9 %). Overall, the hexane extract of chaya have greater hypoglycemic effect, by reducing blood glucose from 126.4 to 105.0 mg/dL (16.9 %) after 6 h, and has no statistical difference with the aqueous, diethyl ether, hexane, acetone and ethyl acetate extracts and glibenclamide ($p>0.05$); at the sixth hour of the experiment the six *C. aconitifolius* extracts reported their lowest concentration on glucose (95.8 – 105.0 mg/dL). Within the second dose of 5 mg/kg, during the first hour the extracts that showed hypoglycemic effect were hexane 125.0 to 117.8 mg/dL (5.8 %), acetone 107.8 – 104.0 mg/dL (3.5 %) and ethyl acetate 108.8 to 105.8 mg/dL (2.8 %). The diethyl ether extract of chaya exhibited hypoglycemic effect until second hour with blood glucose from 120.4 to 118.0 mg/dL (1.7%), meanwhile the aqueous did until the fourth hour from 108.0 to 102.6 mg/dl (5.0 %). The results suggest, the hypoglycemic effect of the hexanoic extract of chaya, by decreasing the blood glucose from 126.4 to 115.4 mg/dL (22.9 %). After six hours, three extracts (hexanoic, diethyl ether and ethyl acetate) had no significant difference respect the positive control ($p > 0.5$).

The terpenes and sterols compounds have shown hypoglycemic effect in different parts of glucose metabolism. Researches have elucidated compounds inside the hexane, ethyl acetate and diethyl ether extracts of chaya (A. et al., 2017; Ajiboye et al., 2018; García-Rodríguez et al., 2014; Schleicher et al., 2016) showing the presence of pentacyclic terpenes. These compounds have showed inhibition activity in the glucose metabolism: 3-O-β-D-glucopyranosyl increased GLUT 4 receptor translocation and, improvement of insulin secretion and pancreatic β-cell function. Additionally, the ursolic acid reduced blood glucose levels and also improves the insulin secretion of the pancreas. Few studies suggested that characteristics such as the hydroxyl present in the C-3 of the structure and the carboxyl of C-28 and C-27 can inhibit the PTB-1B enzyme, responsible to inhibit the β part of the insulin receptor (Furtado et al., 2017; Nazaruk & Borzym-Kluczyk, 2015). Also the aqueous extract of *C. aconitifolius* have exhibit hypoglycemic effect, according to Ramos-Gomez et al., (2016) and Otitolaiye & Asokan, (2016) aqueous extract of *C. aconitifolius* have the presence of phenolic acids such as rosmarinic acid, chlorogenic acid, 4-Hydroxybenzoic acid, cafferic acid and ferulic acid and flavonoids such as catequin, rutin, hesperidin, quercetin, naringenin, quercetin-3-O-glycoside, kaempferol and vanillin, some saponins and alkaloid, have shown activity on the translocation of GLUT 4, this effect have been attributed to the benzene ring and the hydroxyl group present in the chemical structure of the phytochemicals (Emelike & Unegbu, 2015; Li, Yao, & Li, 2017; Otitolaiye & Asokan, 2016).

**Table 4.** Results of the glucose (mg/dL) in the hypoglycemic effect of six extracts of *C. aconitifolius* at two doses 1.0 and 5.0 mg/kg for 6 hours.

| **Extract** | | | | **Time (h)** | | | |
|---|---|---|---|---|---|---|---|
| **(**1.0 mg/kg**)** | **0** | **1** | **2** | **3** | **4** | **5** | **6** |
| **Aqueous** | 115.4 ± 19.5 | 108.4 ± 2.9$^{a,A}$ | 109.8 ± 8.0$^{a,B}$ | 100.4 ± 1.8$^{a,A}$ | 100.4 ± 1.8$^{a,A}$ | 104.2 ± 6.3$^{a,A}$ | 98.0 ± 6.4$^{a,A}$ |
| **Ethanolic** | 113.8 ± 11.5 | 117.6 ± 10.6$^{a,B}$ | 113.6 ± 12.7$^{a,B}$ | 111.6 ± 7.4$^{a,A}$ | 107.0 ± 5.2$^{a,A}$ | 98.0 ± 9.4$^{a,A}$ | 95.8 ± 7.8$^{a,A}$ |
| **Diethyl ether** | 122.4 ± 21.9 | 119.6 ± 3.6$^{a,A}$ | 116.0 ± 7.4$^{a,B}$ | 113.8 ± 7.6$^{a,A}$ | 104.8 ± 8.0$^{a,A}$ | 106.0 ± 9.0$^{a,A}$ | 101.8 ± 9.4$^{a,A}$ |
| **Hexane** | 126.4 ± 15.9 | 115.4 ± 10.3$^{a,A}$ | 116.0 ± 8.0$^{a,A}$ | 113.6 ± 5.8$^{a,A}$ | 111.0 ± 2.7$^{a,A}$ | 103.6 ± 7.2$^{a,A}$ | 105.0 ± 6.6$^{a,A}$ |
| **Ethyl acetate** | 122.6 ± 12.8 | 114.0 ± 4.4$^{a,A}$ | 117.4 ± 4.6$^{a,B}$ | 113.4 ± 4.3$^{a,A}$ | 114.2 ± 5.0$^{a,A}$ | 108.0 ± 8.1$^{a,A}$ | 103.4 ± 9.1$^{a,A}$ |
| **Acetone** | 121.8 ± 10.1 | 117.2 ± 8.6$^{a,A}$ | 117.0 ± 8.1$^{a,B}$ | 113.8 ± 14.0$^{a,A}$ | 103.8 ±3.6$^{a,A}$ | 99.8 ± 8.2$^{a,A}$ | 96.8 ± 8.01$^{a,A}$ |
| **Glibenglamide** | 113.8 ± 4.0 | 55.8 ± 4.8$^{A}$ | 72.6 ± 22.0$^{A}$ | 101.2 ± 10.3$^{A}$ | 107.4 ± 9.7$^{A}$ | 103.0 ± 5.3$^{A}$ | 95.8 ± 9.2$^{A}$ |
| **Extract** | | | | **Time (h)** | | | |
| **(**5.0 mg/kg**)** | **0** | **1** | **2** | **3** | **4** | **5** | **6** |
| **Aqueous** | 108.0 ± 3.4 | 121.2 ± 6.4$^{b,B}$ | 120.4 ± 12.4$^{c,B}$ | 109.6 ± 1.8$^{bc,B}$ | 102.6 ±10.2$^{ab,A}$ | 106.4 ± 9.2$^{b,B}$ | 98.8 ± 6.1$^{b,B}$ |
| **Ethanolic** | 113.8 ± 4.1 | 114.2 ± 4.1$^{a,B}$ | 119.8 ± 4.7$^{bc,B}$ | 119.6 ± 4.0$^{c,B}$ | 117.4 ± 4.3$^{b,B}$ | 112.8 ± 7.0$^{b,B}$ | 105.2 ± 4.1$^{b,B}$ |
| **Diethyl ether** | 120.4 ± 10.9 | 123.2 ± 12.1$^{a,B}$ | 118.0 ± 6.6$^{ab,B}$ | 110.8 ± 6.7$^{ab,A}$ | 112.0 ± 5.3$^{ab,A}$ | 110.4 ± 7.0$^{b,A}$ | 95.6 ± 8.7$^{a,A}$ |
| **Hexane** | 125.0 ± 5.7 | 117.8 ± 7.4$^{a,B}$ | 112.4 ± 4.5$^{a,B}$ | 112.2 ± 6.9$^{a,A}$ | 110.6 ± 8.6$^{a,A}$ | 97.2 ± 2.7$^{a,A}$ | 96.4 ± 4.1$^{a,A}$ |
| **Ethyl acetate** | 108.8 ± 5.6 | 105.8 ± 6.2$^{a,B}$ | 107.4 ± 5.1$^{ab,B}$ | 103.6 ± 6.7$^{ab,A}$ | 104.4 ± 2.7$^{ab,A}$ | 99.0 ± 6.6$^{b,A}$ | 93.4 ± 6.8$^{ab,A}$ |
| **Acetone** | 107.8 ± 6.5 | 104.0 ± 9.1$^{a,B}$ | 110.4 ± 4.2$^{bc,B}$ | 93.6 ± 6.1$^{a,A}$ | 100.2 ± 9.6$^{ab,A}$ | 94.4 ± 6.0$^{ab,A}$ | 96.6 ± 8.7$^{b,B}$ |
| **Glibenclamide** | 113.0 ± 10.8 | 93.2 ± 3.4$^{A}$ | 85.4 ± 4.5$^{A}$ | 97.0 ± 8.9$^{A}$ | 99.4 ± 6.8$^{A}$ | 94.8 ± 7.8$^{A}$ | 93.4 ± 4.2$^{A}$ |

$^{a-c}$Different superscript letters in the same column indicate statistical difference between extracts by One-way ANOVA and Tukey´s multiple range test. $^{A-B}$Different capitals letters in the same column indicate statistical difference between treatment and positive control by One-way ANOVA and Dunnett test. Data expressed as means ± SD, n = 5, ($p<0.05$).

The results reported in this article exhibit differences between *in vitro* and *in vivo* studies due to bioavailability. It has been shown that *in vitro* studies have a controlled system in which the substrate, enzyme, time, temperature and concentrations are controlled. Meanwhile, *in vivo* studies have the bioavailability factor, these depends on the route of administration (oral, intragastric, intraperitoneal) and the metabolism of a compound (drugs, phytochemicals) (Mouhid et al., 2017). Additionally, not all compounds are metabolized the same way, phenolic compounds have a more simplistic mechanism of absorption than lipids (Karas et al., 2016). In the digestion process, only the 48 % of phenolic compounds are digested, while 42 % are absorbed by enterocytes and then transport to hepatocytes and metabolized (Rein et al., 2013). Furthermore, sterols metabolized faster because of the affinity with the cell membrane; they don't need transporters to enhance their permeability; this allows these types of compounds to absorb faster. Overall, extracts with non-soluble compounds can absorb faster and present biological effects faster, than extracts with polar compounds that have to be metabolized more slowly.

## 4. Conclusions

The results showed that all chaya extracts exhibited inhibition of ACE and intestinal enzymes ($\alpha$-amylase and $\alpha$-glucosidase), specially the acetone and ethyl acetate extract. Meanwhile, the *in vivo* results suggested that overall chaya extracts have a hypoglycemic and hypotensive effect, although aqueous and hexane extracts have the greatest effect by lowering blood glucose and blood pressure in rats with obesity, hyperglycemia and hypertension. Thus, the results suggest the biofunctional value of the aqueous extract of chaya, since factors such as yield, bioavailability, accessibility and possible use as a nutraceutical are determining factors in the choice of an extract. Future research is necessary to use the extract as a possible ingredient of functional foods to treat DM and hypertension.

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## 6. Declaration of Interest

The authors report no conflicts of interest.

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## CONCLUSIONES

Los ensayos *in vitro* de la presente investigación ponen de manifiesto que todos los extractos de chaya exhibieron inhibición de la ECA y de las enzimas intestinales ($\alpha$-amilasa y $\alpha$-glucosidasa). El extracto de acetona presentó el menor valor de $CI_{50}$ en la enzima ECA con 12.61 µg/mL. Mientras que, el extracto de acetato de etilo presentó el menor valor de $CI_{50}$ en la enzima $\alpha$-amilasa y $\alpha$-glucosidasa con 22.97 y 3.20 µg/mL, respectivamente.

El estudio *in vivo* sugiere que, todos los extractos de *C. aconitifolius* tienen efecto hipoglucémico e hipotensor en el modelo de ratas obesas con hiperglucemia y HAS. Al administrar el extracto acuoso de *C. aconitifolius* vía peritoneal (5 mg/kg) se observó el mayor efecto hipotensor al disminuir la presión arterial sistólica y diastólica en 15.3 y 23.4%, respectivamente. En la CTG el extracto hexánico a una dosis 0.5 mg/kg tuvo el mayor efecto esperado al disminuir la glucosa en 5.1%. Por su parte, el extracto de acetona de hojas de chaya tuvo el mayor efecto al reducir la glucosa 4.8 % en la CTG a 5.0 mg/kg, ambos extractos después de 30 min. Después, de la administración vía intraperitoneal de los extractos de *C. aconitifolius* en ratas con hiperglucemia, se observó que, el extracto hexánico presentó el mayor efecto hipoglucémico al reducir la glucosa 16.9 y 22.9 % con las dosis de 1.0 y 5.0 mg/kg respectivamente.