Magnesium Rich Extract of Cashew Tree (*Anacardium Occidentale*) Nut and its Principal Compound, Anacardic Acid, Stimulate Glucose Uptake in C2C12 Muscle Cells

Abstract

Magnesium (Mg^{2+}) is the fourth most abundant cation in the whole body and the second most abundant cation within the cell. Numerous cellular functions and enzymes, including ion channels, metabolic cycles, and signaling pathways are regulated by Mg^{2+}. In our previous studies, we found that hydro-ethanolic extract of cashew seed (CSE) at a dose of 25ug/ml, 50ug/ml and 100ug/ml stimulated glucose transport in C2C12 myotubes in a concentration dependant- manner [1]. The stimulation of glucose uptake in skeletal muscle was increased through activation of Adenosine Monophosphate-Activated Protein Kinase (AMPk). These scientific information's on magnesium and diabetes enable us to investigate magnesium content in cashew nut from Cameroon. From the data of the 9 plantations studied, the Cashews Nuts (CN) mean content in Mg was 271 mg/100g wet weight. We did not notice any significant difference between the farms in term of Mg content; remarkably, the Mg content of cashew nut 271mg/100g compared favorably with that of USDA nutrient database (292mg/100g) or Brazilian Mg CN content (277 mg/100 g). The result of this study showed that cashew nut from Garoua-Cameroon is nutritionally riches in term of mineral such as Mg and could be recommended for Magnesium deficient patients.

Keywords: Cashew nuts; Magnesium; Calcium; C2C12 cells; DNA and protein; ICP-MS; Type 2 Diabetes

Research Article

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Abbreviations: AMPk: Adenosine Monophosphate-Activated Protein Kinase; CN: Cashews Nuts; DM: Diabetes Mellitus; CSE : Hydro-Ethanolic Extract of Cashew Seed; WW: Wet Weight; NA: Not Available; AA: Anacardic Acid

Introduction

Dietary magnesium received less attention than dietary calcium by the nutrition research community in the United States during the 20th Century. A number of clinical disorders such as type 2 diabetes and metabolic syndrome, hypertension, atherosclerotic vascular disease, sudden cardiac death and osteoporosis have been associated with a low-magnesium diet. In spite of this, practically half (48%) of the US population has been shown to consume less than the daily magnesium requirement from foods [2].

The seed of cashew nut is one of the fruits most consume worldwide. The seed of cashew is an oleaginous dried fruit in the taste sweeten and magnesium rich nut. It is very rich in unsaturated fatty acids, as the oleic and linoleic, in vitamins B1 and B2 (overtaken by almond nut and Walnut), in panthothenic acid and minerals as the magnesium (260 mg/100 mg), the potassium, the iron and the phosphorus. Its content in Magnesium is one of the higher of the plant kingdom, overtaken only by the sunflower seed (354 mg / 100 g) [3].

The Magnesium intervenes in numerous metabolic functions but especially in the transmission of the nervous impulses. Its deficiency produces the nervousness and the irritability and even the cramps and spasms. Moreover, the magnesium is essential for the nervous stability. Magnesium used is particularly recommended in case of:

I. Abnormal nervousness, nervous irritability, depression, weakness and fatigue.

II. Cramps in the hollow organs: colon, in the womb (dysmenorrhea) or in the coronary arteries of breast [4].

Physiologically, Magnesium (Mg^{2+}) is the fourth most abundant cation in the whole body and the second most abundant cation within the cell. Numerous cellular functions and enzymes, including ion channels, metabolic cycles, and signaling pathways are regulated by Mg^{2+}. At the subcellular level magnesium regulates contractile proteins, modulates trans membrane transport of calcium (Ca^{2+}), sodium (Na^{+}‡) and potassium (K^{+}‡), acts as an essential cofactor in the activation of ATPase, controls metabolic regulation of energy-dependent cytoplasmic and mitochondrial pathways and influences DNA and protein synthesis (Table 1). Mammalian cells regulate Mg^{2+} concentration through specialized influx and efflux transport systems that have only recently been characterized. Magnesium efflux occurs via Na^{+}- dependent and Na^{+} - independent pathways. Mg^{2+}influx is controlled by recently cloned transporters including Mrs2p, SLC41A1, SLC41A1, ACDP2, MagT1, TRPM6 and TRPM7 [5].

Diabetes mellitus (DM) has continued to increase worldwide; in 2013, according to International Diabetes Federation, an estimated 381 million people had diabetes and will increase to 439 million adults by 2030 [6]. The standard therapy for diabetes control includes lifestyle changes (lifestyle counseling,

weight-loss education and exercise), use of oral hypoglycemic drugs, and/or subcutaneous insulin injections. Several studies have demonstrated available glucose-lowering pharmacological agents with their advantages and disadvantages. However, the large number of limitations and unwanted side effects of actual diabetes agents limit their use [7].

Table 1: Magnesium (Mg) – Physiological functions (NIH, 2013).

No.	Magnesium (Mg) – Physiological Functions
1	Contributes to structural development of bones and teeth and is required for DNA and RNA synthesis and the antioxidant glutathione.
2	It helps to maintain normal functioning of skeletal, smooth and cardiac muscle.
3	Sometimes referred to as 'nature's physiological Ca 2+ channel blocker. Like other calcium antagonists it acts as a vasodilator and inhibits coagulation.
4	Reduces neuron excitability. It inhibits Ach release at the neuromuscular junction, and reduces the effect of the excitatory CNS neurotransmitter NMDA.
5	Contributes to normal energy metabolism. Mg plays a role in the active transport of calcium and potassium ions across cell membranes.
6	Helps maintain normal healthy brain function, and in particular, psychological functions
7	Cofactor of more than 300 enzymes systems that regulate protein synthesis and carbohydrate metabolism, muscle and nerve function, blood glucose and blood pressure regulation.

Cashew plant extracts widely used to improve the glucidic and lipidic profiles of patients with diabetes. In our previous studies, we found that hydro-ethanolic extract of cashew seed (CSE), stimulated glucose transport in C2C12 myotubes [1].

Cashew nuts are high in nutrient such as Mg that may improve glucose homeostasis. Hypomagnesaemia is a common feature in patients with diabetes and it might lead to a decrease in insulin mediated glucose uptake and depress release of chemical energy (ATP is form by Mg-ATP oxidative phosphorylation). In the other size Cashew nut is among the 9 top magnesium rich foods (Figure 1). This scientific information on magnesium and diabetes prompted us to investigate magnesium content in cashew nut from Cameroon.

Cashew tree is found in the tropical region of Cameroon (Adamaoua, North and Far-North region). It is a hardy and drought resistant tree thriving in a variety of soil and climatic conditions. The semi-arid north of Cameroon is the hottest and driest part of the country, experiencing average temperatures 25-27°C in the cool seasons (SON, DJF) and 27-30°C in the warmer seasons (MAM, JJA) [8].

About the rainfall, semi-arid regions of Cameroon receive less than 100 mm/mon which is favorable for cashew growing area rainfall [9].

Around the world, and in Cameroon in particular, the name 'Cashew' refers to both the ''apple'' and the kidney-shaped, nutlike seed of the cashew tree. In the north of Cameroon the cashew apple is well consumed during the season while the nut is roasted and consumed in little quantities in the north-Cameroon all year

Figure 1: Magnesium rich food.

round. Before eating, cashew nuts are roasted over open fires or in red hot charcoal in most north rural communities, allowed to cool before they are cracked and consumed. Currently, 75 to 80% of cashew nuts produced in North-Cameroon are intended for consumption, as only very few companies are involved in local processing of the produce.

Materials and Method

Collection of the sample

The sample was collected from 9 plantations around the Benoue River, Graoua-Cameroon. After extraction of the nuts and air dying, the sample were reduced into powder and submitted for Magnesium content analysis (Avomeen Analytical Services) following the described protocol.

Technique

Used for magnesium analysis: Inductible Coupled Plasma/ Mass Spectrometry

Inductively coupled plasma-Mass Spectrometry or ICP-MS is a powerful analytical technique used for multielemental ultratrace analysis of a wide variety of samples. Several people (among users, manufacturers, etc.) consider it to be a routine technique.

It is capable of detecting metals and several non-metals with atomic mass ranges 7 to 250. This is achieved by ionizing the sample with inductively coupled plasma and then using a mass spectrometer to separate and quantify those ions. The technique was commercially introduced in 1983 and has gained general acceptance in many types of laboratories [10].

The benefits of using plasma compared to other ionization methods, such as flame ionization, are that ionization occurs in a chemically inert environment, preventing oxide formation, and ionization is more complete. Also, the temperature profile of the torch is relatively uniform, reducing self-absorption effects. Linear calibration curves are observed over several orders of magnitude for ionization processes. The ability to handle both simple and complex matrices with a minimum of matrix interferences due to the high-temperature of the ICP source Compared to atomic absorption techniques, ICP-MS has greater speed, precision, and sensitivity. However, compared with other types of mass spectrometry, such as TIMS and Glow Discharge, ICP-MS introduces a lot of interfering species: argon from the plasma, component gasses of air that leak through the cone orifices, and contamination from glassware and the cones.

Sample preparation

sample was collected as previously described.

Analysis of the magnesium content was carry out by Inducible Coupled Plasma/Mass Spectrometry and describes as follow

Digestions (via EPA Method SW846 3050): 1 gram of sample was weighted in 50 mL digestion tube. To the sample in the tube, 6 mL of concentrated Nitric Acid and 2.5 mL of concentrated Hydrochloric Acid was added. The sample was covered with a glass watch glass and heated for 1 hour in a hot block at 95ºC. The sample was then removed from the hot block and allowed to cool. Then 3 mL of 30% Hydrogen peroxide was added to the sample and heated on the hot block for an additional 30 minutes. Sample was removed from the hot block, raised up to 50 mL final volume with deionized water and analyzed by ICP-MS. This technique was used following its benefits mentioned in 2.2 paragraph.

Analysis (via EPA Method SW846 6020): Inductively Coupled Plasma-Mass Spectrometer Analysis (EPA Method SW846 6020)

Inductively Coupled Argon Plasma Mass Spectrometer: Agilent 7500CE

Mass Flow Controller, Babington Nebulizer, water cooled quartz spray chamber, quartz torch inside 4-turn load coil, Nickel cones, 3 sets of lenses (extraction, Einzel lens assembly and omega lens block assembly), 2 sets of quadrapole rods and an electron multiplier detector, Peristaltic Pump, Octa-pole/reaction cell technology with helium, hydrogen and no gas modes to eliminate most interferences.

- Argon Gas Supply: UHP grade supplied at 100PSIG
- Helium and Hydrogen Cylinders
- Water Chiller: 15ºC + 1ºC
- Cetac Autosampler: Models ASX and ASX-520
- Computer System: Chemware software
- Instrument is allowed to become thermally stable before beginning prior to calibration.

Results and Discussion

Of the 27 Cashew samples collected from 9 farms, moisture content was measured for 6 and Mg analysis for all on a wet weight basis. Analyses were performed by Avomeen Analytical Services, Ann Arbor, MI 48108 USA. Mg analysis was performed by Inductively Coupled Plasma Mass Spectrometry/EPA SW846 6020 and moisture analysis was by AOAC 934.01, 17th edn. (2000).

The mean Mg content of these cashew seeds was 271 mg Mg/100 g wet weight (WW), comparing closely with the value for Cashews in the USDA nutrient database* (Table 1). The Cameroon cashews were higher in moisture content than those reported by the USDA and a bit lower in Mg content for both dry and wet weight bases. However, the USDA report contains only one study of unknown sample size, and does not report any variance so a true comparison is not possible.

Table 2 Percentage moisture and Mg content, wet weight (WW) basis, for cashews from 9 farms in Cameroon compared with cashews reported is USDA database.

Table 2: Magnesium composition of cashew nut. Nine farms (n=3).

Cashew Source	n	% Moisture Mean (SD)	% Moisture Range	Mg Content WW Mg/100g Mean (Sd)	Mg Content WW Mg/100g Range
Cameroon, 6 Spls with % Moisture Measured	6	6.4% (0.630)	6.0-7.7	253 (22.51)	230 - 270
Cameroon, all Samples	27	6.4% Assumed	NA	271 (30.04)	210 - 320
USDA*	1 study n Unknown	5.2% (NA)	5.09 - 5.32	292 (NA)	289 - 295

NA: not available; WW: wet weight

The proximate compositions of the cashew nut magnesium content studied are shown in (Table 1). From the data of the nine farms (Fa) studied, it was observed that the cashew nut mean content in magnesium was 271 mg/100g. There was no significant difference between farms in term of magnesium content. Remarkably, the magnesium content of cashew nut 271/100g compared favorably with the USDA nutrient database (292 mg/100g) [11]. The maximum magnesium (300 mg/100g)

content was noted in samples collected around the Benoue River. This variation could be explained by the altitude which is considered as physical factors on cashew yield production. The variation of magnesium content could also be explained by the soil samples analysis, unfortunately, the latter was not done. Physiologically, magnesium has various important functions; it is a cofactor of DNA and protein synthesis, for oxidative phosphorylation and for many enzymes, it is a co-factor for more than 300 different metabolic reactions, particularly those involved in energy storage. Magnesium also functions as a calcium antagonist and is required for neuromuscular excitability [12]. Mg is one of the trace elements that have enjoyed numerous experiments owing to its involvement with insulin sensitivity and carbohydrate metabolism and other vital physiological functions. There exists a positive link between hypomagnesaemia and hyperglycemia. The cause of diabetic hypomagnesaemia is multifactorial and diabetic patients have significantly lower mean serum Mg levels compared with healthy counterparts [13].

In the other hand, a meta-analysis study demonstrated that magnesium intake is inversely associated with incidence of type 2 diabetes. This finding also suggests that increased consumption of magnesium-rich foods such as whole grains, beans, nuts, and green leafy vegetables may reduce the risk of type 2 diabetes [14].

In our previous study, we showed that hydro-ethanolic extract of cashew tree (*Anacardium occidentale*) nut and its principal compound, anacardic acid, stimulate glucose uptake in C2C12 (Figure 2) muscle in a concentration-dependent manner. The ability of tree nuts to improve glycemic control may relate to activation of adenosine monophosphate-activated protein kinase in C2C12 myotubes as we demonstrated [1]. Our results are in line with a recent meta-analysis of randomized controlled dietary trials supporting the effect of tree nut on glycemic control in diabetes [15].

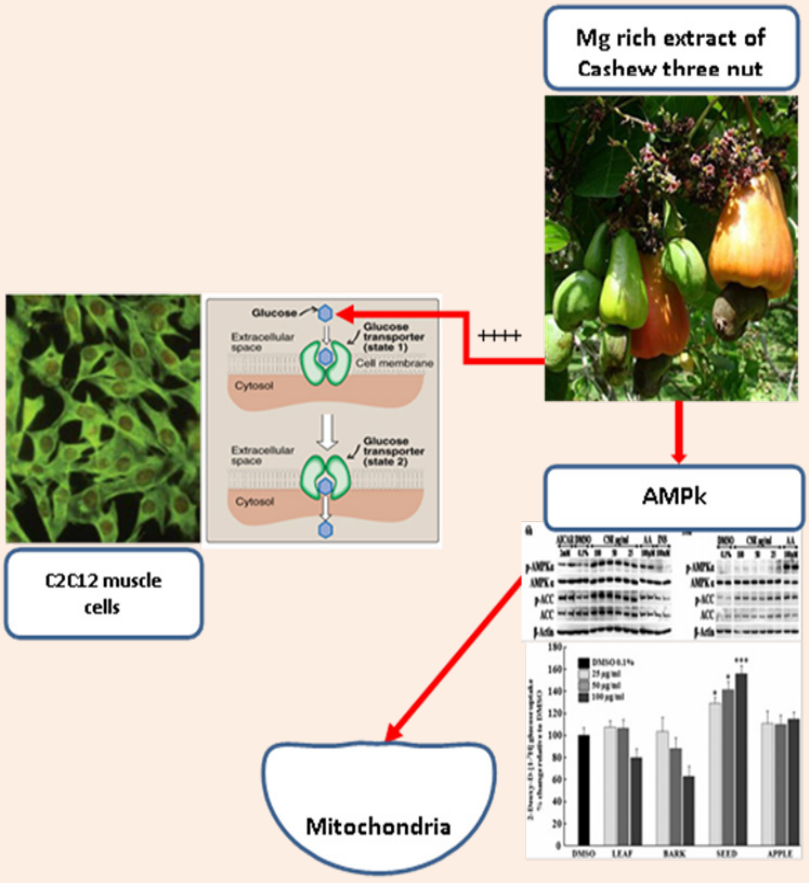

Figure 2: Effect of Anacardium occidentale hydroethanolic extracts plants parts on Glucose uptake in C2C12 myotubes [1].

The other proposed factors for glycemic control relate to micro nutriment profile of nuts including magnesium. Magnesium content of cashew nut from Garoua-Cameroon ranged between 271 - 300 mg /100 g, with 100 g of cashew nuts thus providing 73% of the daily value of magnesium. Moreover, higher dietary intake of magnesium from food plus supplements is associated with significantly reduced odds ratios for elevated glycohemoglobin, metabolic syndrome, obesity, overweight or obesity, elevated waist circumference, elevated systolic blood pressure, reduced HDL and elevated C-reactive protein. Thus, there is a beneficial relationship between dietary magnesium intake and diabetes-related physiological outcomes in U.S. adults [16].

About the moisture content, we analyzed 6 of 17 samples (35%) and the mean moisture content was 6.42%. About the identify parameter, we neither observe any huge variation nor significant different between the 6 moisture samples. Moisture is

important to investigate, for low moisture contents can inhibit the growth of microorganisms and cause minimal changes in texture. According to Soares et al. [17], the very moist nut becomes elastic and is little appreciated by consumers. Moisture can be important to the storage period for farmers, and thus an important factor that can affect yield of cashew. This happens in the critical period June to October in which moisture is important for cashew.

Concluding Remarks

The result of this study showed that cashew nut from Garoua-Cameroon is nutritionally riches in term of mineral such as Magnesium. In our previous study we demonstrated that cashews seed extract (CSE) and it principal compound anacardic acid (AA) stimulates glucose in C2C12 cells myotubes in a concentration dependent-manner. Activation of adenosine monophosphate-activated protein kinase by CSE and AA likely increases plasma membrane glucose transporters, resulting in elevated glucose uptake. The present study pointed out that the ability of cashews nut to improve glucose transport may relate to micronutrient such as magnesium taking into account it content in the cashew's nut and it role on diabetes equilibrium. Nevertheless, the high magnesium content in the cashew nut showed its potential to prevent depletion for children and adults. This study supports the dietary recommendation to increase consumption of major food sources of magnesium, such cashew nuts.

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