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Comparative study of antihypertensive and antioxidant effects of clove and metformin on renal dysfunction in streptozotocin-induced diabetic rats

Guenzet Akila, Krouf Djamil*, Dida Nawal, Berzou Saadia

Laboratory of Clinical and Metabolic Nutrition, Department of Biology, Faculty of Nature and Life Sciences, University of Oran 1 Ahmed Benbella, BP 1524 EL M'Naouer, 31000, Oran, Algeria

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Streptomycin sulfate (PubChem CID: 19648)
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Titanium (IV) oxysulfate-sulfuric acid (PubChem CID: 10866624)

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ABSTRACT

The effect of exposure to diabetes on the kidney appears to be modulated by elevated level of oxidative stress and increased blood pressure. We used streptozotocin-induced diabetes in rats to further explore the importance of renal dysfunction associated with oxidative status and hypertension and the modulatory anti-hyperglycemic effects of clove in comparison with metformin against kidney injury. Diabetes was induced intraperitonially by a single injection of streptozotocin (55 mg/kg bw). One untreated diabetic group (D) consumed 20% casein and drinks only water, whereas the two other groups consumed the same diet and received either *Syzygium aromaticum* extract dissolved in water (D-*Sa*) by gavage or a glucophage MTF dissolved in water (D-MTF), for 4 weeks. The injection of streptozotocin in rats leads to increases blood pressure, alteration in the redox state of the kidney and electrolytic imbalance. However, administration of clove or metformin reduces blood pressure, serum sodium and increases potassium level. Histology analysis revealed several glomerular and tubule-interstitial alterations were effectively reduced by treatment with both clove and metformin. The renoprotective effect induced by these drugs was due to modulation of oxidative status. The present study, thereby demonstrates the hypotensive and renoprotective effects of clove.

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from either an absolute or relative deficiency of insulin secretion or action [1]. One of the most important complications of this metabolic disease is the diabetic nephropathy, which contributes to high blood pressure [2]. Several previous investigations have confirmed the role of oxidative stress in developmental diabetic nephropathy [3], possibly by oxygen free radical formation [4], in which oxidative stress cause increased accumulation of advanced glycated end products in the kidney of diabetic patients [5]. Experimental studies have consistently reported that hyperglycemia can affect renal function by increasing renin angiotensin system activity [6], causing more reabsorption of sodium [7]. However, these alterations of electrolytes may play a vital role in diabetic nephropathy leading to hypertension [8]. Most of the studies reveal the inference of oxidative stress in diabetes pathogenesis by augmenting arachidonic acid oxidation and formation of vasoconstrictive prostaglandins [9]. 8-Iso-prostaglandin a major F2-isoprostane is not only an indicator of oxidative stress but also a potent renal vasoconstrictor [10]. Therefore, a broad derangement in nonenzymatic biochemistry involving both lipids and carbohydrates exists in diabetic glomerular lesions [11]. Protein oxidation may, therefore, represent an important factor in the development of symptoms in diabetic patients [12]. In the other hand, hyperglycemia overwhelms the enzymatic systems defense include [superoxide dismutase (SOD), an enzyme that inactivates superoxide radicals, and catalase (CAT), an enzyme responsible for the removal of H_2O_2] and impaired glutathione metabolism [13]. Thus, besides controlling weight and hyperglycemia, reduction of blood pressure and oxidative stress is an efficient way of slowing the progression of nephropathy disorders in clinical treatment of diabetes [14]. A popular oral drug for treating diabetes, metformin is a member of a class of drugs called biguanides that helps lower blood glucose levels by improving the way the body handles insulin [15]. Furthermore, it has

Abbreviations: Sa, Syzygium aromaticum; STZ, streptozotocin; 8Iso-PGF2α, F2-isoprostanes; BP, blood pressure; C, control group; CAT, catalase; GFR, glomerular filtration rate; GSH, reduced glutathione; H&E, hematoxylin and eosin; HbA1c, glycosylated haemoglobin; K^+, potassium ion; LPO, lipid hydroperoxides; Na^+, sodium ion; SOD, superoxide dismutase

* Corresponding author.
E-mail address: djamil@lncm-univoran.com (K. Djamil).

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been reported that glucophage, a widely used metformin, not only lowers blood glucose levels, which in the long term reduces the risk of diabetic complications, but it also appears to have antioxidant properties [16], could play an important role in preventing nephropathy disorders. In the same way, a substantial body of evidence suggests that plant extracts with hypoglycemic properties had a range of important pharmacological properties that may retard the progressive decline in renal function in diabetes [17]. However, spices which have been known to improve the sensory properties of foods have also been appreciated for their medicinal values. Clove bud (*Syzygium aromaticum L.*) Is an aromatic flower bud belonging to the family of Myrtaceae, it has a deep brown color, intense fragrance and burning taste. Commonly used in Africa in preparation of various spicy, rich dishes [18] and they are consumed as whole spices or ground into powder and mixed with diets containing cereals [19]. In addition to its culinary uses, the clove bud and its oil have an abundance of medicinal and recreational uses. Clove has been identified as a hypoglycemic food adjunct in both laboratory animals and human experimental protocols [20,21]. For instance, it has been reported that clove bud diet has antihyperglycemic, hypolipidemic, hepatoprotective and antioxidative properties in the type 2 diabetic condition [22]. However, the favorable effects of aqueous extract of clove buds of kidney injury in streptozotocin induced type 1diabetic rats have not been elucidated. Consequently, the present study examined the importance of renal dysfunction associated with oxidative stress and hypertension and the modulatory effects of clove in comparison with metformin against kidney injury through the approaches of using STZ-induced diabetes in rats.

2. Materials and methods

2.1. Plant material

Dried clove buds *Syzygium aromaticum* (L) Merr. and Perry were purchased from the local market of Oran, Algeria. Buds were powdered with the help of the grinder and clove extract was prepared as follows: 50 g of the powdered buds was refluxed at 60–70 °C in 500 ml distillated water for 30 min and the decoction was filtered with cotton wool. The filtrate was concentrated at 65 °C by a rotavapor (BuchiLabortechnink AG, Postfach, Switzerland) under a reduced pressure and frozen at −70 °C before lyophilization (Christ, alpha 1–2 LD). The crude yield of the lyophilized extract was approximately 23% (wt/wt). It was stored at ambient temperature until further use.

2.2. Animals

Male Wistar rats (Pasteur Institute, Algiers, Algeria), weighing 285 ± 10 g were housed under standard environmental conditions (23 ± 1 °C, 55 ± 5% humidity and a 12 h light/dark cycle) and maintained with free access to water and a casein diet *ad libitum*. The composition of casein diet (expressed in g/kg) was: casein, 200 (95% purity, Prolabo, Paris, France); sunflower oil, 50; sucrose, 50; cellulose, 50; cornstarch, 600; minerals 40; vitamins, 20 (Merck, Darmstadt, Germany). The general guidelines for the care and use of laboratory animals recommended by the Council of European Communities [23] were followed and all the experimental protocols involving the use of laboratory animals were approved by the Institutional Animal Ethics Committee of Oran 1 ABB University (Reg. No. 13/355/2015).

Diabetes was induced by intraperitoneal injection of streptozotocin (STZ) (Sigma, St Louis, Mo, USA) at a dose of 55 mg/kg bw STZ was dissolved in 0.05 mol/l cold sodium citrate buffer, pH 4.5 immediately before use. After 48 h, hyperglycemia was confirmed using a strips test, Glucometer (ACCU-CHEK Active, Roche Diagnostics, Mannheim, Germany). Only animals with fasting blood glucose levels greater than 16 mmol/l were considered diabetic and then included in this study.

2.3. Diet and treatments

Diabetic rats (n = 24) were divided into three groups and fed for 4 weeks a casein diet. The untreated group (D) only received water, whereas the treated group received daily orally through gavage, *Syzygium aromaticum* extract (D-*Sa*) (2 g/kg bw) or glucophage as a reference compound (D-MTF) at 350 mg/kg bw. A normal rats (control group: C, n = 8) was injected with 0.25 ml of cold sodium citrate buffer and was fed a casein diet during the experiment. Blood pressure measurements of animals were recorded one every week and blood glucose levels were also measured weekly as described above. At the end of the study period, all rats are subjected to 24-h urine collection.

After the 4 weeks of the experiment, the rats were fasted overnight and anesthetized with chloral hydrate 10% (3 ml/kg bw) and then bled from the abdominal aorta in tubes. Blood was collected into dried tubes and serum was prepared by low speed centrifugation (1000*g* for 20 min at 4 °C). The kidneys were removed immediately, rinsed with cold saline, and weighed. A small section of this tissue was placed in 10% neutral buffered formalin for histological evaluation. Aliquots of serum and kidney were stored at −70 °C until analyzed.

2.4. Blood pressure

Blood pressure (BP) is measured in the tail of rat using tail cuff System (CODA 4; Kent Scientific Corporation, USA). The CODA 4-Channel system measures the blood pressure up to 4 rats simultaneously. This tail-cuff system uses volume pressure recording to measure the blood pressure by determining the tail blood volume. To adapt the animal to the holder and cuffs, an acclimation period is usually recommended by placing the animal in the holder for 15 min for 3 consecutive days prior to the actual study. Thirty minutes before the measurements, the rats were placed into a preheated restrainer 32 °C, with the tail exposed. The tail cuff was pushed up to the base of the tail and fit closely but freely on the tail and the pulse sensor was placed just behind the tail cuff. The cuff was then inflated and deflated automatically during periods of 90 s during 20 min. The pressure in the occlusion cuff and the pulse signal was monitored and recorded in a PowerLab/400 (software Chart for Windows) system.

2.5. Blood and urine parameters

Serum Insulin was analyzed using an enzyme immunoassay kit based on the competition between unlabeled rat insulin and acetyl cholinesterase linked to rat insulin (tracer) for limited specific guinea-pig anti-rat insulin antiserum sites (Spi-Bio, Le Bretonneux, France). Glycosylated haemoglobin (HbA1c) was estimated by ion exchange chromatography method (KitBiocon, Germany). Quantitative measurements of sodium ion (Na^+) was estimated by Mg-Uranyl acetate method and that of potassium ion (K^+) was performed using tetraphenylboron-Na method (Kits Chronolab, Barcelona Spain).

Kidney function parameters including urea and creatinine were measured in serum by enzymatic colorimetric methods (Kits Spinreact, Girona Spain). Glomerular filtration rate (GFR), as assessed by creatinine clearance from measurements of urinary and serum concentrations of creatinine and urine flow rate in the 4th week.

2.6. Histological analysis

Hematoxylin and eosin (H&E) staining was performed to reveal the morphological features of the tissue [24]. Kidney samples for histology were obtained after proper fixation with formalin then dehydrated in ascending grades of ethyl alcohol, and subsequently embedded in paraffin blocks. Slices with 5 µm sections were stained with (H&E) and investigated under bright field Leitz microscope (Leitz Wetzlar, LEICA).