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Research Article
Lemon Peels (*Citrus latifolia*, *Rutaceae*) Compared to Garlic Cloves (*Allium Sativum L.*) has the Highest Antioxidant Activity "*In vitro*" and Protect Against Free Radical Attack in Streptozotocin-induced Diabetic Wistar Rats

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Abstract

Background and Objective: Lemon and garlic have been used for a long time in traditional medicine. This "*in vitro*" study aimed to screen hydro-methanolic extract (HME) of garlic gloves (*gc*) and lemon peels (*lp*) and evaluate their effect on oxidative stress, lipid profile and glycaemia in diabetic rats. **Materials and Methods:** After preparation HME of two plants and performs a phytochemical screening, 24 male wistar rats were divided in four groups. Control group (C) received citrate buffer intraperitoneal. The other groups received Streptozotocin (40 mg kg^{-1} BW, ip) injection to induce diabetes. One diabetic group (D) was untreated, the two other groups were treated by lp extract (D-*lp*) or gc extract (D-*gc*) (200 mg kg^{-1} BW/Day). **Results:** The "*in vitro*" study showed that *lp*HME compared to gcHME were richer in total polyphenols, flavonoids and condensed tannins and has a better DPPH radical scavenging action and anti-hemolytic potential. The "*in vivo*" study showed that in D-*gc* compared to D-*lp* and D groups, body weight was increased significantly and intraperitoneal glucose tolerance was improved. At Day 28, glycaemia was reduced significantly in D-*gc* and D-*lp* compared to D group. In diabetic rats, *lp* decreased total cholesterol, LDL-cholesterol and triacylglycerols. Antioxidant study showed that TBARS levels were significantly reduced in D-*lp* and D-*gc* groups in kidney. Therefore, Kidney glutathione values increased in D-*gc* compared to D-*lp* groups. **Conclusion:** Results suggested that feeding *lp* improved redox status, lipid profile and glycaemia. Also, *gc* decreased glycaemia, glucose tolerance and antioxidant defense. Both plants reduced diabetes complications by different mechanisms of action.

Key words: Antioxidant activity, diabetes, garlic cloves, lemon peels, phenolic compounds, rats

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes is a serious chronic condition associated with metabolic disorders characterized by increased glycaemia. The prevalence of diabetes has been increasing worldwide. In 2013, it affected 382 million people and caused 5.1 million deaths. A total of 316 million people have a low glucose tolerance and are at high risk from the disease, which is expected to reach 471 million by 2035[1].

Several studies have revealed that insulin resistance and diabetes are characterized by dyslipidemia, a major risk factor for cardiovascular disease. The disturbance of lipid metabolism leads to diabetic dyslipidemia, an early event in type 2 diabetes patients by several years[2]. Dyslipidemia is characterized by increased total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triacylglycerols (TAG) levels and lipid ratio parameter [LDL-C to high-density lipoprotein cholesterol (HDL-C) ratio] and decreased HDL-C[3].

Diabetic complications are often triggered by hyperglycemia-induced oxidative stress[4]. On the other hand, insulin resistance, dyslipidemia, β-cell dysfunction and impaired glucose tolerance are caused by oxidative stress[4]. Many studies demonstrated that patients with diabetes experience higher levels of oxidative stress due to increased production of reactive oxygen species and also have reduced antioxidant defenses mechanisms[2]. In diabetes pathogenesis, oxidative stress increases lipid peroxidation by increasing thiobarbituric acid reactants and decreasing antioxidant enzyme activity[5] and glutathione levels[6].

Several components of fruits and vegetables such as garlic[7] or lemon[8] have anti-diabetic[7] and antioxidant effects[7,8]. Polyphenols or flavonoids are among these components[9]. There are many studies that have been conducted regarding flavonoids and their bioactive properties, for example, flavonoids have been associated with a reduction in diabetes and obesity[10].

The objective of this *in vitro* study was two-fold. Firstly, it attempted to investigate and compare total phenolic and flavonoid contents and antioxidant activity of garlic cloves and lemon peels hydro-methanolic extracts (HME). Secondly, using streptozotocin-induced diabetic Wistar rats, this "*in vivo*" study compared the effect of these two extracts (garlic clovesand lemon peels) on glycaemia, serum lipids and antioxidant activity.

MATERIALS AND METHODS

Preparation of hydro-methanolic extracts from garlic cloves and lemon peels powders: The garlic (*Allium sativum L.*, *Liliaceae*) and lemon (*Citrus latifolia*, *Rutaceae*) plants were identified taxonomically and authenticated by the Botanical Research Laboratory of Oran University. The two plants were purchased from a local market. To obtain garlic cloves powder (*gc*) or lemon peels powder (*lp*), plant material was grated and dried in an oven for 24 h at 38° C and grounded into powder and stored at 4 °C until use.

To extract phenolic compounds, methanol (80%, 10 mL) and distilled water (1: 1, v/v) were added to 10 g of powder from each plant. The mixture was left to macerate with stirring for 24 hrs at room temperature. The extraction was repeated 3 times. The extracts were collected and centrifuged at 3000 g for 10 min and filtered through filter paper. The solvent was removed under vacuum at 45 °C using a Rotavapor[11]. After calculating the extraction yield (41% for lemon peels and 35% for garlic cloves), 10 mL of methanol (80%) had recovered 1 g of the dry residue from each plant and stored at -20 °C for *in vitro* study and 100 mL of distilled water had recovered 15 g of the dry residue from each plant (15 mg mL^{-1}) for *in vivo* study.

In vitro study:

Total polyphenols levels in hydro-methanolic extracts (HME):

The total polyphenols of HME contents of the two plants powders were determined with Folin-Ciocalteu reagent according to the method of Singleton and Rossi[12]. Folin-Ciocalteu's reagent was a yellow acid consisting of a mixture of phosphotungstic acid and phosphomolybdic acid, which was reduced during the oxidation of phenols, to a mixture of blue oxides of tungsten and molybdenum. The coloration produced, whose maximum absorption at 760 nm, is proportional to the quantity of polyphenols in the plant extracts.

Only 0.2 mL of extract was mixed with 1 mL of Folin Ciocalteu reagent and made up to 20 mL with 4.25% bicarbonate (CO_3Na_2) solution. Under the same conditions, a control was prepared with distilled water. All samples were placed in a water bath at +70 °C for 20 min. After cooling, the absorbance of the samples was performed at 760 nm relative to control (performed in triplicate). A calibration curve was prepared before and under the same conditions, using gallic acid as a reference. The concentration of polyphenols in the samples was estimated by referring to the calibration curve of gallic acid. The results were expressed in mg of Gallic Acid Equivalents (GAE) in 100 g of dry weight (DW) plant matter (mg GAE 100 g^{-1} DW).

Total flavonoid content in hydro-methanolic extracts: Flavonoid content was determined as described by Bahorun *et al.*[13]. The quercetin calibration curve was prepared

by mixing 1 mL of samples and quercetin methanolic solution with 1 mL of aluminium trichloride solution (2% in methanol). After 10 min, absorbance was measured at 415 nm (performed in triplicate). A standard range of Quercetin in µg mL^{-1} serves as a reference for the determination of the flavonoid content. The results were expressed as mg of quercetin equivalents (QE) per gram of dry weight plant matter (mg QE 100 g^{-1} DW).

Condensed tannins content: Evaluation of condensed tannins content (CTC) was performed by Chupin *et al.*[14] method. The extracts (1 mL) were placed in two test tubes (sample and blank). Methanol vanillin solution (4%, w/v) (3 mL) and 1.5 mL concentrated hydrochloric acid were added to the sample tube, while 3 mL pure methanol and 1.5 mL concentrated hydrochloric acid were added to the blank tube. After 15 min of incubation in dark room, the absorbance was measured at 500 nm (performed in triplicate). A standard curve was prepared with catechin (0.5-8 mg mL^{-1}). The results were expressed as milligrams of catechin equivalents (CE) per gram of dry weight (mg CE g^{-1} DW).

DPPH free radical scavenging activity: The DPPH (2.2 diphenyl 1 picrylhydrazyl) radical scavenging activity of the extracts was determined as cited by Banerjee *et al.*[15]. Antioxidants in samples can give hydrogen (such as hydroxyl groups in phenolic compounds) will reduce DPPH which is a free radical, stable and acceptor of hydrogen. The reduction in DPPH is accompanied by the change of the color of the solution from the violet to the yellow with a maximum absorbance at 517 nm. Afterward, 75 µL of DPPH (3 mM, 1.3 mg mL^{-1} of methanol) added to 3mL extract from each plant was incubated in the dark for one hour. Then a negative control containing only methanol was prepared. The absorbance was read at 517 nm.

Percentage of inhibition activity was calculated using the following equation:

$$\text{I DPPH (\%)} = 1\text{-(Abs control-Abs sample)}\times 100$$

Each set of experiments was performed in triplicate:

- **I DPPH (%):** Percentage of inhibition of the DPPH radical
- **Abs sample:** Absorbance of the sample
- **Abs control:** Absorbance of the negative control

Anti-hemolytic potential: The anti-hemolytic potential (AHP) of the plants was determined as described by Singh and Kaur[16]. Fresh heparinized blood was obtained from a healthy volunteer subject and then was centrifuged at 1500 g for 10 min at 4°C. After removal of the serum, the erythrocyte pellet was washed with phosphate-buffered saline (PBS, 0.2 M, pH: 7.4) until the supernatant became clear. After this, 2500 µL of the erythrocytes suspension was added with 500 µL of the hydro-methanolic extract (10 mg mL^{-1}) of each plant and incubated at 37°C for 1 hr with shaking. Then 500 µL of the mixture were taken every 15 min (15, 30, 45 and 60 min) and mixed with 1.5 mL of PBS and centrifuged at 2400 rpm for 10 min, then the absorbance at 548 nm against a blank (PBS solution) was performed for different times.

A control solution (free of extract) or total hemolysis was prepared under the same experimental conditions by adding 500 µL H_2O_2, 30%. The absorbance of supernatants was noted at 540 nm. The relative hemolysis was assessed in comparison with the total hemolysis solution, which was set as 100%. Each set of experiments was performed in triplicate.

Anti-hemolytic potential was calculated and expressed as percent inhibition of hemolysis relative to the total hemolysis after one hour of incubation according to following equation:

$$\text{Hemolysis rate (\%)} = 1\text{-(Abs control-Abs sample)}\times 100$$

- **Abs sample:** Absorbance of sample
- **Abs control:** Absorbance of control

In vivo study:

Animals and dietary treatments: The General Guidelines on the Use of Living Animals in Scientific Investigations[17] were followed and all animal experiments were performed in compliance with the protocol approved by our institutional committee on animal care and use. A total of 30 male Wistar rats (Iffa Credo, L'Arbresle, Lyon, France) weighing 250±10 g were housed in stainless steel cages at 24°C with a 12 hrs light/dark cycle and 60% relative humidity. Body weight (BW) and glycaemia of rats were determined weekly.

Animals were fed a standard-diet that included casein, corn starch, vegetable oil, cellulose, mineral and vitamin mixtures.

Streptozotocin-induced diabetic rats: After overnight fasting (n = 24), diabetes was induced by intraperitoneal injection of a single dose of Streptozotocin STZ [Sigma-Aldrich, St. Louis, USA, in citrate buffer (0.1 M, pH 4.5)] at 40 mg kg^{-1} BW[18]. Then 72 hrs after the STZ injection, glycaemia was measured in codal vein blood by strips test using a portable glucometer (One Call Extra, ACON laboratories, San Diego. USA). Animals (n = 18) with fasting glycaemia ≥7.2 mmol L^{-1} (130 mg dL^{-1}) were considered diabetic.

After two days of acclimatization, the experimental diabetic rats (D), were randomly divided into 3 groups of six rats, the first untreated group (D) was force-fed with distilled

water (2 mL/rat/day) serving as a positive control, the second group (D-*gc*) was treated with the HME of garlic cloves (*gc*) (200 mg kg^{-1} BW/days) and the third group (D-*lp*) was treated with the HME of lemon peels (*lp*). And finally a fourth group (C) of non-diabetic rats (n = 6) received distilled water (2 mL/rat/Day) serving as a negative control.

Intraperitoneal glucose tolerance test (IPGTT): Rats were fasted for 12 hrs and injected with a 40% glucose solution prepared in distilled water (2 g kg^{-1} BW). Glycaemia was determined using the glucometer (One Call Extra) in tail vein blood before (at 0 min) and after (at 15, 30, 60 and 120 min) the injection of glucose solution.

Blood and tissue samples: After 4 weeks of the experiment, the rats were fasted overnight and anesthetized with chloral hydrate 10% (3 mL kg^{-1} BW) and euthanized with an overdose. Blood was obtained from the abdominal aorta and collected in the tubes containing ethylene diamine tetra acetic acid-Na_2 (EDTA) (Sigma, St Louis, Mo). Blood serum was centrifuged at low-speed for 20 min at 1000 g at 4°C. The liver, heart and kidney were removed, rinsed with cold saline and weighed. Aliquots of serum and of each tissue were stored at -70°C until analyzed.

Determination glycemia and lipid profile in blood: Fasting glycaemia was determined using glucometer. Serum levels of triacylglycerol (TAG), total cholesterol (TC), High Density Lipoproteins cholesterol (HDL-C) and Low Density Lipoproteins cholesterol (LDL-C) were measured by an enzymatic colorimetric test (kit, Biocon).

Serum and tissues thiobarbituric acid reactive substances (TBARS) assay: The determination of thiobarbituric acid reactive substances (TBARS) was carried out by measuring malondialdehyde (MDA), the main marker for the determination of free radicals and degradation products of lipid peroxides following exposure to oxidative stress. This method is based on the formation in an acidic and hot between MDA and two molecules of TBA (Thiobarbituric Acid, Sigma-Aldrich Chemie, Germany) of an absorbent pigment (chromogenic condensation complex) at 532 nm, extractable by organic solvent (butanol). The MDA concentration was determined from MDA standard curve (Sigma-Aldrich Chemie, Germany).

Serum TBARS assay: The levels of serum TBARS were determined as described by Quintanilha *et al.*[19]. After dilution of 100 µL of each sample in 0.9 mL of NaCl (0.9%), 20 µL of butylated hydroxytoluene (BHT) (2% BHT in ethanol) (Sigma-Aldrich Chemie, Germany) and 1 mL of TBA (15% TCA and 0.375% TBA in 0.5N HCl) were added. Samples were incubated at 85°C for 30 min and cooled in ice and centrifuged at 2000 g for 10 min, at 4°C. The samples were read by spectrophotometry at λ = 532nm. The results were expressed as µmol of MDA dL^{-1} serum.

Tissues TBARS assay: The lipid peroxidation in tissues was assessed by the complex formed between malondialdehyde and thiobarbituric acid (TBA)[20]. The liver, heart and kidney (0.5 g) were homogenized with 4.5 mL of KCl (1.15%). The homogenate (100 mL) was mixed with 0.1 mL of sodium dodecyl sulfate (8.1%), 750 mL of acetic acid (20 %) and 750 mL of TBA reagent (0.8%). The reaction mixture was heated for 60min at 95°C. After cooling, 2.5 mL of n-butanol pyridine (15:1) was added at each sample, mixed and centrifuged at 4000 g for 10 min, the upper phase was taken for measurement at λ = 532 nm. The results were expressed as µmol of MDA g^{-1} of tissue.

Erythrocyte and tissue reduced glutathione (GSH) assay: Reduced glutathione was determined as described by Sedlak and Lindsay[21]. The tissue homogenates were prepared in phosphate buffer (0.1 M pH = 6). Then, 500 µL of sample (tissue homogenates or erythrocyte lysate) were mixed with 400 µL of cold distilled water and 100 µL of 50% TCA, after shaking the samples were centrifuged at 1200 g, for 15 min. Then, 500 µL of supernatant were mixed with 400 µL of 0.4 M Tris buffer (pH 8.9) and 100 µL of 0.01 M DTNB (5.5′-dithiobis [2-nitrobenzoic acid]). After 5 min of incubation, the absorbance was read at λ = 412 nm against a reagent blank. A stock solution of 10 mM reduced glutathione (Sigma Chemical Company, St. Louis, MO, USA) was used to establish the standard curve. The results were expressed in µmol mL^{-1} for erythrocytes and in µmol g^{-1} for tissues.

Statistical analyses: All data were presented as Means ± SDs of 3 tests per extract (*in vitro* study) or of six rats per group (*in vivo* study). Data were analyzed using two-way analysis of variance (ANOVA) followed by post-hoc Tukey honestly signi cant difference test using the STATISTICA (version 4.1; Statsoft, Tulska, Okla).

RESULTS

***In vitro* study:** The extraction yield of the *lp*-HME and *gc*-HME were 41 and 35%, respectively. Polyphenols, flavonoids and tannins contents significantly increased by +74%, + 54% and

Table 1: Phenolic content, antioxidant and anti-hemolytic activities of garlic cloves and lemon peels hydro-methanolic extracts

	HME garlic cloves	HME lemon peel
Total phenolic compounds (mg GAE 100 g^{-1} DW)	4.60±0.24	17.92±0.95***
Flavonoid content (mg QE 100 g^{-1} DW)	2.22±0.057	4.88±0.53**
Condensed tannins content (mg CE g^{-1} DW)	3.40±0.05	6.30±2.5**
Inhibition of the DPPH radical (%)	84.00±2	97.00±1*
Hemolysis rate (%)	73.82±0.14	81.59±0,07**

Data are presented as Means±SDs and analyzed by two-way ANOVA followed by Tukey, *p<0.05, **p<0.01, ***p<0.001, DW: Dry weight, GAE: Galic acid equivalents, QE: Quercetin equivalents, CE: Catechin equivalents, HME: Hydro-methanolic extracts

Table 2: Body weight (BW), glycaemia and Intraperitoneal glucose tolerance test (IPGTT) of rats

	Control	Diabetics	Diabetic-HME garlic cloves	Diabetic-HME lemon peels
Body weight (g)				
Day 0	240.00±13	240.00±26	242.00±7	246.67±11
Day 7	279.33±5	196.00±19**	262.75±17##	242.00±7**##$$
Day 14	299.50±12	211.67±25**	275.25±19##	235.67±21**1$$
Day 21	312.37±14	214.67±17**	279.00 ± 14##	231.00±19**$$
Day 28	307.00±23	217.67±2**	281.25±16##	243.67±26**$$
Glycaemia (mg dL^{-1})				
Day 0	96.670±5	208.33±40**	246.67±21**	214.50±8**
Day 7	59.330±17	203.40±19**	142.00±17**##	270.00±18**##$$
Day 14	85.670±5	142.75±31**	103.33±22#	133.33±14*
Day 21	74.330±10	159.20±35**	138.67±12**	124.33±43*
Day 28	76.000±14	146.83±11**	126.33±15**#	121.00±5**##
IPGT test (mg dL^{-1})				
0 min	78 .330±12	166.60±47**	126.00±19*	127.50±30*
15 min	103.33±30	240.80±50**	126.75±54##	300.00±45**$$
30 min	112.00±43	222.60±20**	130.50±11##	279.50±12**##$$
60 min	79.670±20	187.60±24**	124.25±7**##	216.00±3**$$
120 min	66.000±10	188.00±19**	150.25±26**#	179.50±5**

Data are presented as Means±SDs and analyzed by two-way ANOVA followed by Tukey post-hoc test, *p<0.05 vs Control, †p<0.05 vs Diabetics, ‡p<0.05 vs diabetic-HME lemon peels, **p<0.05 vs Control, † †p<0.05 vs diabetics, ‡ ‡p<0.05 vs Diabetic-HME lemon peels, HME: Hydro-methanolic extracts, Control: Control rats treated with distilled water (2 mL/rat/day), Diabetics: Diabetics rats treated with distilled water (2 mL/rat/day), Diabetics-HME garlic cloves: Diabetics rats treated with HME of garlic cloves (*gc*) (200 mg kg^{-1} BW/days), Diabetics-HME lemon peels: Diabetics rats treated with the HME of lemon peels (*lp*) (200 mg kg^{-1} BW/days), IPGT: Intraperitoneal glucose tolerance

+46% in the *lp*-HME compared to *gc*-HME, respectively (Table 1). The *lp*-HME compared to *gc*-HME increased DPPH radical scavenging capacity (+13%) and antihemolytic activity (+10%) (Table 1).

***In vivo* study:** Body weight of rats significantly increased by +11 and +25% at day 7, +14 and +23% at day 14, +17 and +23% at day 21, +13 and +22% at day 28, in D-*gc* group compared to D-*lp* and D groups, respectively (Table 2).

Fasting glycaemia decreased in D-*gc* group compared to D-*lp* and D groups, by -34 and -30% at Day 7. However, at day 14 and 28 glycaemia decreased by -12 and -17% in D-gc group compared to D group, respectively. Likewise at Day 28, glycaemia decreased by -17% and -14% in D-*gc* and D-*lp* groups compared to D group, respectively (Table 2).

The IPGTT showed a reduction of glycaemia in D-*gc* group compared to D and D-*lp* groups by -47 and -20% at 15 min, by -41 and -53% at 30 min and by -33 and -42% at 60 min, respectively. However, at 120 min glycaemia decreased significantly by -20% in D-*gc* compared to D group (p<0.01) (Table 2).

The serum lipid profile showed a reduction of TC by -32%, -58 and -53%, in D-*lp* group compared to C, D and D-*gc* groups, respectively and of LDL-C by -68 and -70% in D-*lp* group compared to D and D-*gc* groups, respectively (p<0.05) (Table 3).

Likewise, the TAG values decreased by -74 and -52% in D-*lp* compared to D and D-*gc* groups. Furthermore, HDL-C decrease by -45, -47 and -42% in D, D-*gc* and D-*lp* groups compared to control group, respectively (p<0.05) (Table 3).

Lipid peroxidation showed increment in serum TBARS values in D group compared to C, D-*gc* and D-*lp* groups by +89, +90 and +82%, respectively. Inversely, they were decreased significantly in D-*lp* group compared to D and D-*gc* groups in kidney by -87 and -61% and in liver by -76 and -50%, respectively (p<0.01) (Table 4).

Table 3: Lipid profile of rats

mg dL^{-1}	Control	Diabetics	Diabetic-HME garlic cloves	Diabetic-HME lemon peels
TC	131.83±14	215.00±20**	192.08±3**#	89.67±12**##$$
HDL-C	61.200±14	33.580±5**	31.850±3**	35.42±2**
LDL-C	55.380±17	116.72±23**	125.39±5**	37,45±14##$$
TAG	103.90±23	323.45±35**	174.22±5**##	84.00±20##$$

Data are presented as Means±SDs and analyzed by two-way ANOVA followed by Tukey post-hoc test, *p<0.05 vs Control, †p<0.05 vs diabetics, ‡p<0.05 vs Diabetic-HME lemon peels, **p<0.05 vs control, ††p<0.05 vs Diabetics, ‡‡p<0.05 vs diabetic-HME lemon peels. HME: Hydro-methanolic extracts, Control: Control rats treated with distilled water (2 mL/rat/day), Diabetics: Diabetics rats treated with distilled water (2 mL/rat/day), Diabetics-HME garlic cloves: Diabetics rats treated with HME of garlic cloves (*gc*) (200 mg kg^{-1} BW/days), Diabetics-HME lemon peels: Diabetics rats treated with the HME of lemon peels (*lp*) (200 mg kg^{-1} BW/days)

Table 4: Blood and tissues TBARS (MDA: malondialdehyde) and glutathione values of rats

	Control	Diabetics	Diabetic-HME garlic cloves	Diabetic-HME lemon peels
TBARS				
Serum (µmol dL^{-1})	6.22±1.8	56.77±11**	5.40±0.5††	9.82±0.4††
Kidney (MDA µmol g^{-1} tissue)	0.27±0.07	2.500±0.07**	0.98±0.10**†††	0.32±0.2††‡‡
Liver (MDA µmol g^{-1} tissue)	0.40±0.04	2.870±0.4**	1.44±0.2**††	0.68±0.28††‡‡
Reduced glutathione				
Erythrocytes (µmol mL^{-1})	0.54±0.10	0.850±0.12**	0.73±0.09	0.55±0.15††
Kidney (µmol g^{-1} tissue)	0.57±0.10	0.530±0.07	0.75±0.14*††	0.12±0.03**††‡‡
Liver (µmol g^{-1} tissue)	0.36±0.13	0.500±0.13	0.57±0.21	0.49±0.06

Data are presented as Means±SDs and analyzed by two-way ANOVA followed by Tukey post-hoc test, *p<0.05 vs control, †p<0.05 vs Diabetics, ‡p<0.05 vs diabetic-HME lemon peels, **p<0.05 vs control, ††p<0.05 vs Diabetics, ‡‡p<0.05 vs diabetic-HME lemon peels. HME: Hydro-methanolic extracts, Control: Control rats treated with distilled water (2 mL rat^{-1}/day), Diabetics: Diabetics rats treated with distilled water (2 mL/rat/day), diabetics-HME garlic cloves: Diabetics rats treated with HME of garlic cloves (*gc*) (200 mg kg^{-1} BW/days), Diabetics-HME lemon peels: Diabetics rats treated with the HME of lemon peels (*lp*) (200 mg kg^{-1} BW/days)

Erythrocytes glutathione values were decreased by -35 and -36% in D-*lp* and C groups compared to D group. Also, in kidney glutathione values were decreased in D-*gc* compared to C, D and D-*lp* groups by +24%, +29% and +84%, respectively (p<0.01) (Table 4).

DISCUSSION

***In vitro* study:** The "*in vitro*" study showed that *lp*-HME is richer in polyphenols, flavonoids and condensed tannins and presented a greater antioxidant activity than *gc*-HME. However, *gc*-HME seems to protect better against hemolysis than lp-HME (Table 1). Polyphenolic compounds are generally present in peels of citrus fruits and protect the inner tissues from harmful UV and IR rays of sun and from microbial infections[22]. After comparison between some studies, we have found that lemon peels contains more phenolic compounds than garlic cloves. Lemon aurantifolia peels have maximum polyphenol (34.59±0.81 mg GAE g^{-1} DW) and flavonoid contents (23.06±1.57 mg QE g^{-1} DW)[8]. However, garlic cloves showed lower values than those of the lemon peels. The contents of total phenolic compounds in selected garlic cultivation had mean value of 6.5 mg GAE g^{-1} DW[23].

The most important group of bioactive compounds in citrus limon fruit are flavonoids such as flavonones (hesperidin, hesperetin, naringin); flavones (diosmin, orientin and vitexin) and flavonols (quercetin; limocitrin and spinacetin). In comparison to another Citrus species, citrus limon has the highest content of eriocitrin[24]. The citrus lemon peel contains quercetin and kaempferol[25].

However, the major active components of garlic are organosulfur compounds, such as diallyl thiosulfonate (*Allicin*), diallyl sul de, diallyl disul de, diallyl trisul de, S-allyl-cysteine and S-allyl-cysteine sulfoxide (*Alliin*)[26]. Moreover, the main phenolic compounds in garlic are resorcylic acid, pyrogallol, gallic acid, rutin, protocatechuic acid and quercetin[27].

Some previous studies agree with the results of the present study, for example, Makni *et al.*[28] showed a DPPH-scavenging activity of 80% in lemon peel extract. However, Nencini *et al.*[29] showed a weaker DPPH-scavenging activity (45.63%) in garlic cloves. Some studies revealed that plant extracts have antihaemolytic effect, probably due to polyphenolic compounds, for example the Mallotus fruit extract (Euphorbiaceae family plant)[30]. In addition, polyphenols are known to chelate transition metals such as Fe^{2+}, thus reducing the rate of the Fenton reaction. They can also prevent oxidation caused by the hydroxyl radical and prevent the passage of H_2O_2 through the erythrocyte membrane and the free radicals generation[31,32].

The *gc*-HME protect against hemolysis due to the action of Organic polysulfides (Diallyl Disulfide and Diallyl Trisulfide) which act as H_2S donors[32]. H_2S is a physiological mediator able

to limit inflammation and free radical damage by reacting with multiple oxidant stressors, as hydrogen peroxide and superoxide radical anion[32]. The studies cited above reveal a difference in the composition of bioactive compounds between garlic and lemon, which may explain the activity of different antioxidant and antihemolytic.

***In vivo* study:** The "*in vivo*" study showed that diabetic rats treated with *gc*-HME (D-*cg*) revealed an increase in BW and an improvement in glucose tolerance compared to diabetic rats treated with *lp*-HME (D-*lp*). However, both plants extract significantly reduced glycaemia compared to untreated diabetic rats (D) (Table 2).

Results of the present study agree with findings of Martha *et al.*[7] who reported that diabetic rats treated with garlic extract revealed a significant increase in body weight[7]. In diabetes, there is a loss of muscle mass and tissue proteins, resulting in a decrease in body weight[33]. It seems that garlic inhibits this process.

Nobiletin (citrus avonoid) has adipocyte di erentiation inhibitory activity and reduce the obesity correlated with type 2 diabetes and decrease body weight in animals treated with *lp*-HME. has antidiabetic action[10]. In complete cellular lysates, Nobiletin has antidiabetic activity and enhanced Akt phosphorylation and glucose transporter-(GLUT)-1 expression as well as GLUT-4 expression in adipose tissue and muscle plasma membranes. Martha *et al.*[7] observed a significant decrease in glycaemia and increase in insulin in diabetic rats treated with garlic extract (300 or 600 mg kg^{-1} BW/Day). In D-*gc* group, flavonoids in garlic cloves, such as quercetin and their glycosides reduced glycaemia in rat skeletal muscle cells (L6 myotubes) by inhibiting intestinal glucosidase, increasing GLUT-4 translocation, glucose uptake and insulin action[34]. *Allium sativum* organosulfur compounds and flavonoids (quercetin and its glycosides) decrease postprandial blood glucose and inhibit intestinal glucosidase and increase GLUT-4 translocation, glucose uptake and insulin action[34]. On the other hand, other experimental studies carried out on lemon peel reveal an antihyperglycemic effect[35].

Mohanapriya *et al.*[36] reported that ethanol extracts from C. limon peel administered orally (400 mg kg^{-1} daily for 12 days) to diabetic rats reduced blood glucose. This could be due to the action of the phenolic compounds it contains. Pari and Srinivasan[37] observed that oral treatment with Diosmin in diabetic rats signi cantly reduced glycemia level and enhanced the activity of hexokinase and glucose-6-phosphate dehydrogenase (G6PD).

The lipid profile (TAG, CT and LDL-C) showed a significant reduction in rats treated with lemon peel HME (D-*lp*) compared to rats treated with garlic cloves HME (D-*gc*) or untreated diabetic rats (D). Likewise, improved lipid profile was observed in D-gc group compared to D group. Although, both plants improved lipid profile but lemon peel seems to have a stronger effect. This improvement in lipid parameters may be due to the polyphenol composition of lemon peels. For example, some previous studies have shown that Hesperidin, another compound of lemon, has both hypoglycemic and hypolipidemic effects and reduced serum TAG, TC and LDL - VLDL-C concentrations and increase serum HDL-C concentration and HDL/TC ratio, in STZ-diabetic rat[38,39]. Similarly, *Allium sativum*, organosulfur compounds and flavonoids decreased serum cholesterol and TAG and LDL-C [7]. *"In vivo"* evaluation of oxidative stress revealed that lemon peels reduced lipids peroxidation (TBARS values) in kidney and liver compared to garlic cloves. However, a decrease was observed in TBARS values in blood and tissue (kidney, liver) of diabetic rats treated with the two plants compared to untreated diabetic rats. Inversely, garlic cloves increased glutathione values in kidney compared to lemon peels (Table 4).

Our *"in vitro*" study revealed that lemon peels is richer in phenolic compounds than garlic, which can give it better protection against radical attack.

Several studies have shown that lemon peels and these phenolic compounds increased antioxidant activity. For example, Ali *et al.*[39] noted that treatment of diabetic rats with C. reticulata fruit peel extract (hesperidin and quercetin) induced a significant improvement in glutathione content, glutathione peroxidase, glutathione-S-transferase and Superoxide dismutase activities, in liver. The administration of Citrus limetta fruit peel extract (200 and 400 mg kg^{-1} BW.) decreased pancreatic, hepatic and kidney TBARS levels and increased the GSH level in the liver and kidney of STZ-induced rats[40].

Parhiz *et al.*[41] reported that the antioxidant activity of the flavonoids from C. limon (hesperidinand hesperetin) was not only limited to their radical scavenging activity but also augmented the antioxidant cellular defenses via the ERK/Nrf2 signaling pathway. The activation of nuclear transcription factor-erythroid 2-related factor 2 (Nrf2) and release the Nrf2 from Kelch-like ECH-associated protein 1 (Keap1)-Nrf2 complex, involved in the induction of gene encoding detoxifying and antioxidant enzymes[41].

The increase in kidney and erythrocyte glutathione levels and the decrease in TBARS values in D-*gc* group compared to D group, observed in the current study could be due to garlic organosulfur compounds. Garlic organosulfur compounds are spontaneously derived from allicin after cutting of the garlic cloves and are the principal active ingredients that are responsible for the bene cial effects of the garlic extracts.

Garlic organosulfur compounds and their conjugates are also able to release H_2S in a non-enzymatic reaction with intracellular GSH[32]. Garlic Organic polysulfides such as Diallyl disulfide and Diallyl trisulfide act as H_2S donors when they react with biological thiols such as GSH and glucose is necessary to maintain the GSH pool, likely via the pentose phosphate pathway-mediated NADPH production that supports GSH reductase activity and increase glutathione disulfide concentrations in mitochondria of humans red blood cells[32].

CONCLUSION

This "*in vitro*" study showed that *lp*-HME is richer in phenolic compounds than *gc*-HME and seems to have the most important antioxidant activity, however *gc*-HME is richer in organ sulfur compounds and is better protected against hemolysis. On the other hand, the "*in vivo*" study showed that garlic cloves improved body weight and glucose tolerance better than lemon peels. However, in diabetic rats, lemon peels appear to be more protective against free radicals attack and to improve lipid profile than garlic cloves. Both plants help to reduce diabetes complications by different mechanism of action.

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