



Received on 25 April 2014; received in revised form, 26 July 2014; accepted, 08 August 2014; published 01 November 2014

ZYGOPHYLLUM GAETULUM AQUEOUS EXTRACT PROTECTS AGAINST DIABETIC DYSLIPIDEMIA AND ATTENUATES LIVER AND KIDNEY OXIDATIVE DAMAGE IN STREPTOZOTOCIN INDUCED-DIABETIC RATS

Akila Guenzet, Djamil Krouf *, Samira Zennaki and Sadia Berzou

Laboratory of Clinical and Metabolic Nutrition, Department of Biology, Faculty of Natural and Life Sciences, University of Oran, Oran, Algeria.

Keywords:

Rats, Diabetes,
Zygophyllum gaetulum, LCAT,
PON1, Antioxidant enzymes

Correspondence to Author:

Krouf Djamil

Laboratory of Clinical and Metabolic Nutrition, Department of Biology, Faculty of Natural and Life Sciences, University of Oran, Oran, Algeria.

E-mail: lnckrouf@gmail.com

ABSTRACT: *Zygophyllum gaetulum* is one of several traditional remedies used for diabetes treatment in Algeria. The purpose of this study is to investigate the effects of this plant on hyperglycemia, insulin sensitivity indexes, lipid profiles, lecithin: cholesterol acyltransferase (LCAT) and paraoxonase (PON1) activities and liver and kidney antioxidant status, in streptozotocin-induced diabetic rat. Diabetes was induced intraperitoneally by a single injection of streptozotocin (STZ) (55mg/kg bw). Diabetic rats (n=12), weighing 263±5g, were randomly divided into two groups fed a casein diet supplemented or not with *Zg* extract (1g/kg bw), for 4 weeks. The control group (n=6) received 0.23-0.25 ml of citrate buffer and was fed a standard diet during the experiment. Phytochemical analyses of *Zg* revealed the presence of tannins and flavonoids. At d28, in *Zg*-treated vs untreated diabetic rats, glycemia and HOMA-IR were decreased by 58 and 57%, respectively. Inversely, the QUICKI index was increased (+10%). The levels of serum total cholesterol, LDL-HDL₁-cholesterol, triacylglycerols, VLDL-triacylglycerols, and phospholipids were decreased significantly ($p<0.05$). PON1 and LCAT activities were increased by 43%. Thiobarbituric acid reactive substances (TBARS) levels in the liver and kidney were respectively 1.8- and 1.6-fold lower, while the activities of superoxide dismutases (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSSH-Red) and catalase (CAT) in *Zg*-treated rat were enhanced. In conclusion, in streptozotocin-induced diabetic rats, treatment with *Zygophyllum gaetulum* extract reduces glycemia, serum lipids and stimulates PON1 and LCAT activities. In addition, it reduces oxidative stress in the liver and kidney by decreasing TBARS levels and increasing antioxidant enzyme activities.

INTRODUCTION: Diabetes mellitus (DM) is one of the major global health and economic problem, characterized by high levels of blood glucose resulting from defects in insulin production, insulin action, or both ¹.

DM is associated with an elevated level of oxidative stress, alteration of antioxidant enzymes and lipid peroxides production, increased susceptibility to coronary heart disease and reductions in lecithin: cholesterol acyltransferase (LCAT) ² and paraoxonase activities (PON) ³.

LCAT and PON are two of the major enzymes that contribute to the continuous remodeling process of plasma lipoproteins. The LCAT reaction is the source of the majority of cholesteryl ester (CE) in plasma ⁴ and plays a central role in the reverse cholesterol transport (RCT) process ⁵.

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.5(11).4709-17</p>
<p>This article can be accessed online on www.ijpsr.com</p>	
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(11).4709-17</p>	

PON is closely associated with high-density lipoprotein (HDL)-containing apolipoprotein A-I (apo A-I) ⁶ and confer antioxidant properties to HDL. Altered carbohydrate metabolism during diabetes is also accompanied by disordered fat and plasma lipoprotein metabolism ⁷, results in non-enzymatic glycation of plasma proteins, including apo A-I, the most abundant; the in HDL. Glycation affects the structure of apoA-I and its ability to activate LCAT, the key enzyme in the reverse cholesterol transport ⁸. Moreover, LCAT is glycosylated and oxidized as a result of chronic hyperglycemia, the mechanism of paraoxonase reduction in oxidative stress status is not clearly known. However, it is suspected that ROS overproduction leads to increased deactivation of paraoxonase ⁹.

Herbal medicines have a good curative effect on certain diseases, especially for diabetes mellitus which needs continuous medication throughout the life ¹⁰. In the present day, allopathic medicines are costlier and have more side effects which could cause severe damages to the vital organs. Hence, finding a suitable herbal medicine for diabetes is very important in the current situation. In the course of our research program on medicinal plants, we investigated *Zygophyllum gaetulum*; locally named "Agaya" is a succulent plant belonging to the *Zygophyllaceae* family ¹¹ and it is a, growing in the South of Algeria in the basin of Adrar. Folk medicine uses a variety of species of *Zygophyllum* for therapeutic treatment. It was reported that different parts of *Zygophyllum* are used against diabetes, and as a drug active against rheumatism, gout, asthma and hypertension ¹². Moreover, this plant has several antioxidative components, including flavonoids, which exhibit antioxidant properties ¹³. The aqueous extracts of *Zygophyllum gaetulum* (Zg) show no cytotoxicity or genotoxicity and has been used as a condiment in food ¹⁴. Hence, the present study was designed to assess the effects of the Zg lyophilized aqueous extract on glycemia, lipid profiles, LCAT and PON1 activities, liver and kidney oxidative stress, in streptozotocin-induced diabetic rat.

MATERIAL AND METHODS:

Plant Material and Preparation of Extract:

Zygophyllum gaetulum was collected in Southern of Algeria (Adrar), between March and April 2012,

identified taxonomically and authenticated by the Botanical Research Institute of Oran University (voucher specimen number Zg 1965). The plant material was stored at room temperature in a dry place before use. Fresh aerial parts (leaves) of the plant were dried at ambient temperature (24 °C) for 7 days and ground to a powder. The Zg extract was prepared as follows: 50 g of the powdered aerial parts was refluxed at 60-70 °C in 500 ml distillate water for 30 minutes, and the decoction was filtered with cotton wool. The filtrate was concentrated at 65°C by a rotavapor (Buchi Labortechnik AG, Postfach, Switzerland) under reduced pressure and frozen at -70 °C before lyophilization (Christ, alpha 1-2 LD). The crude yield of the lyophilized extract was approximately 30% (wt/wt). It was stored at ambient temperature until further use.

Determination of Total Phenol Compounds:

Total polyphenols content from the extract was quantified using Folin-Ciocalteu's method ¹⁵. Plant extract (200 µl) was mixed with 1 ml of Folin-Ciocalteu reagent (diluted 10%) and incubated at room temperature. After 4 min, 800 µl of sodium carbonate (7.5%) was added. All tests were performed six times. The absorbance was measured after 2 h at 760 nm. The total phenol content was expressed as gallic acid equivalent (GAE) in mg/100 g of dry weight (dw).

Total Flavonoids Contents:

Total flavonoids contents were determined using the method described by Djeridane *et al* ¹⁶. 1 ml of sample was mixed with 1 ml of 2% Aluminum chloride and it was kept at room temperature for 10 min. All tests were performed six times. The absorbance of the reaction mixture was measured at 430 nm and the total flavonoid content was expressed as Quercetin equivalent (QE) in mg/100 g dw.

Estimation of Tannin Concentrations:

The protein precipitation method was used for the determination of tannin concentration as described by Hagerman and Butler ¹⁷. 20 µl of the sample extract was diluted with distilled water (2%) and 250 mg bovine serum albumin (BSA) was added, mixed and incubated for 30 min at 4 °C. Then, the sample was centrifuged for 15 min at 2000g and the supernatant was retained. Tannins contents determination of the supernatant sample was

carried out according to the same procedure described previously¹⁵. All tests were performed six times. The tannin content was subtracted from the total phenol values and was expressed as gallic acid equivalent (GAE) in mg/100 g dw.

Diphenyl-picryl-hydrazyl Radical Scavenging (DPPH) Assay:

The value of the lowest IC₅₀ of extract expresses the strongest activity of this extract. The free radical scavenging capacity of the aqueous extract of *Z. gaetulum* was tested by its ability to bleach the stable 2, 2-diphenyl 2-picryl-hydrazyl (DPPH) radical¹⁸. A stock solution of DPPH (1.5 mg/ml of methanol) was prepared such that 75 µl of it in 3 ml methanol gave initial absorbance of 0.9. This stock solution was used to measure the antiradical activity.

The decrease in absorbance in the presence of aqueous extract of *Z. gaetulum* at different concentrations (concentrations of stock solutions were 4 to 25 g/L) was measured after 30 min. IC₅₀ was calculated from percentage inhibition. Ascorbic acid was used as reference standard. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh¹⁹:

$$PI = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

The value of the lowest IC₅₀ of extract expresses the strongest activity of this extract.

Ferric Reducing Antioxidant Power (FRAP) Assay:

The FRAP assay measures the change in absorbance at 593 nm due to the formation of a blue colored complex of ferrous ion (Fe²⁺) and 2,4,6-tripyridyl-striazine (TPTZ). Prior to this, colorless ferric ion (Fe³⁺) was oxidized to ferrous ion (Fe²⁺) by the action of the electron-donating antioxidants. This assay has been described by Katalinic *et al.*²⁰ In fact, freshly prepared FRAP reagent was warmed at 37 °C in a water bath. This reagent was prepared by mixing 10 mM of TPTZ in 40 mM HCl, 20 mM FeCl₃ and 0.3 M acetate buffer (pH 3.6) in the ratio of (1:1:10). An aliquot of 25 µl of the extract was added to 475 µl of FRAP reagent. After 30 min of incubation at 37 °C, the absorbance of the reaction mixture was measured. All tests were performed six times. Gallic acid was used as a reference to produce a

standard curve and all results were expressed as milligram gallic acid equivalent (GAE) in mg/100 g dw.

Experimental Animals and Drug Administration:

Male Wistar rats (Iffa Credo, I' Arbresle, Lyon, France), weighing 263 ± 5g 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 standard diet *ad libitum*. The ingredient composition of the diet (expressed in g/kg) was: casein, 200 (95% purity, Prolabo, Paris, France), sunflower oil, 50; sucrose, 40; cellulose, 50; cornstarch, 590; minerals 40; vitamins, 20. Food and tap water were provided *ad libitum*. The General Guidelines on the Use of Living Animals in Scientific Investigations²¹ were followed, and the protocol and use of rats were approved by our institutional committee for animal care and use. After one week of acclimatization, rats were subjected to a 12 h fasting. Diabetes was induced by a single intraperitoneal injection of freshly STZ (Sigma, St. Louis, MO, USA) dissolved in citrate buffer (0.01M, pH 4.5), at a dose of 55 mg/kg body weight. Forty-eight hours after STZ administration, the diabetic state was assessed by measuring glycemia using a one-touch glucometer (Accu-Chek Active, Germany). Only rats in which fasting blood glucose levels greater than 16 mmol/L within 3 days after STZ injection were considered diabetic. STZ-diabetic rats were randomly divided into two groups (n=12) and received diet containing 20% casein supplemented (*Zg*-treated) or not (untreated diabetic) with *Zg* extract (1 g/kg BW), for 28 days. The control group (n=6) received 0.23-0.25 ml of citrate buffer and was fed a standard diet during the experiment.

Preparation of Blood and Tissue Samples:

After the 4 weeks of the experiment, the rats were fasted overnight and anesthetized with chloral hydrate 10% (3 ml/kg bw) and euthanized with an overdose. Blood was obtained from the abdominal aorta of rats and collected into tubes containing ethylenediaminetetraacetic acid-Na₂ (Sigma, St Louis, Mo). Blood plasma was prepared by low-speed centrifugation at 1000g for 20 minutes at 4 °C. Liver and kidney were removed immediately, rinsed with cold saline, and weighed. Aliquots of

plasma and 50 to 100 mg of each tissue were stored at -70 °C until analyzed.

Lipoprotein Separation: Serum VLDLs and LDL-HDL₁ were isolated by precipitation using MgCl₂ and phosphotungstate (Sigma Chemical Company, France) by the method of Burstein *et al.*²²

Biochemical Estimations: Blood glucose levels were determined as described above. Insulin was measured using an enzyme immunoassay kit based on the competition between unlabeled rat insulin and acetylcholinesterase linked to rat insulin (tracer) for limited specific Guinea-pig anti-rat insulin antiserum sites (enzyme immunoassay kit; Spi-Bio, Le Bretonneux, France). The color intensity was determined by spectrophotometer at 405nm. Glycosylated hemoglobin (HbA_{1c}) was estimated by ion-exchange chromatography method (Kit Biocon, Germany). Serum apo A-I concentrations were determined by immunoturbidimetric method (kit Orion Diagnostica, Spain). Total cholesterol (TC), triacylglycerol (TG) and phospholipids (PL) of serum and each fraction were determined with the enzymatic method (Kits Spinreact, Girona Spain) Uric acid and albumin levels were measured by enzymatic colorimetric methods (Kits Spinreact, Girona Spain). Alanin aminotransferase (ALT; EC 2.6.1.1) and aspartate aminotransferase (AST; EC 2.6.1.2) were determined by kinetic colorimetric assay (Kits Biocon, Germany).

Insulin Resistance Markers: The quantitative insulin sensitivity check index (QUICKI) was calculated as previously defined from fasting glucose and insulin values²³. QUICKI as $1/(\log \text{fasting glucose [mg/dL]} + \log \text{fasting insulin } [\mu\text{U/mL}])$. The Homeostasis Model Assessment Insulin Resistance Index (Homa-IR) was calculated using the following formula: $\text{Homa-IR} = [\text{fasting insulin } (\mu\text{U/mL}) \times \text{glucose (mmol/L)}]/22.5$. The Homa- β index, which defines the function of the beta cell, was calculated by the formula $\text{Homa-}\beta = 20 \times \text{insulin } (\mu\text{U/mL}) / (\text{glucose (mmol/L)})$ ²⁴.

Lecithin: Cholesterol Acyltransferase (LCAT) Activity Assay: Serum LCAT activity was assayed according to the method of Glomset and Wright²⁵ modified by Knipping²⁶. Cholesterol and egg phosphatidylcholines (PC) were used for the

preparation of liposomes. Specifically, 2 mg cholesterol and 16 mg egg PC in chloroform-methanol (2:1, v/v) were evaporated to dryness under nitrogen stream. After adding 1.0 ml of (10 mM Tris-HCl, 150 mM NaCl and 1.0 mM EDTA, pH 7.4), the solution was sonicated for 30 min at 100 W and 25 °C. LCAT activity was determined using liposomes as a substrate. The cholesterol esterifying activity was expressed as nanomoles of esterified cholesterol/h/ml of serum.

Paraoxonase (PON1) Activity Assay: Serum PON1 activity was estimated spectrophotometrically by the method of Schiavon *et al.*²⁷ Briefly, the assay mixture consisted of 500 μl of 2.2 mmol/l paraoxone substrate in 0.1 mol/l Tris-HCl buffer, pH 8, containing 2mmol/L CaCl₂ and 50 μl of fresh serum. The absorbance was monitored at 405 nm, at 25 °C. The PON1 activity was expressed in international units (IU). One IU was defined as 1 μmol of p-nitrophenol which was formed/min/L.

Measurement of the Lipid Peroxidation and Antioxidant Enzyme Activities: The Lipid peroxidation in tissues was assessed by the complex formed between malondialdehyde (MDA) and thiobarbituric acid (TBA)²⁸. Briefly, the liver and kidney (0.5 g) were homogenized with 4.5 ml of KCl (1.15%). The homogenate (100 μl) was mixed with 0.1 ml of sodium dodecyl sulfate (8.1%), 750 μl of acetic acid (20%), and 750 μl of TBA reagent (0.8%). The reaction mixture was heated at 95°C for 60 min. After heating, the tubes were cooled, and 2.5 ml of n-butanol-pyridine (15:1) was added. After mixing and centrifugation at 4000g for 10 minutes, the upper phase was taken for measurement at 532 nm.

Superoxide Dismutase (SOD; EC 1.15.1.1): It is a metalloenzyme that catalyzes the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide. Superoxide dismutase assay uses a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine (kit; Cayman). One unit of SOD was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. One gram of tissue was homogenized in 5 to 10 ml of cold 20 mmol/l N-2 hydroxyethyl piperazine-N'-2-ethane sulfonic acid buffer, pH 7.2, containing 1

mmol/l ethylene glycol-bis (2-aminoethoxy)-tetraacetic acid, 210 mmol/l mannitol, and 70 mmol/l sucrose. The homogenate was centrifuged at 1500g for 5 minutes at 4°C, the supernatant was removed, and SOD activity was measured at 440 to 460 nm using a plate reader.

Glutathione peroxidase (GSH-Px; EC 1.11.1.9):

Catalyzes the reduction of hydroperoxides, including hydrogen peroxide, by reduced glutathione and functions to protect the cell from oxidative damage. Glutathione peroxidase assay measures GSH-Px activity indirectly by a coupled reaction with glutathione reductase (kit; Cayman). The oxidation of NADPH to NADP⁺ was accompanied by a decrease in absorbance at 340 nm. One unit of GSH-Px was defined as the amount of enzyme that catalyzed the oxidation of 1nmol of NADPH per minute at 25 °C. One gram of tissue was homogenized in cold buffer (50 mmol/l Tris - HCl, pH 7.5, 5 mmol/l ethylenediamine tetra acetic acid, and 1 mmol/l 1, 4-dithiothreitol). The homogenate was centrifuged at 10 000g for 15 min at 4 °C. The supernatant containing GSH-Px was removed and measured at 340 nm using a plate reader.

Glutathione Reductase (GSSH-Red; EC 1.6.4.2):

Activity was evaluated at 340 nm by measuring the decrease in NADPH absorbance in the presence of oxidized glutathione ²⁹. One unit of enzyme

reduces 1 μmol oxidized glutathione per min at pH 7 at 25 °C.

Catalase (CAT; EC 1.11.1.6): Catalyzes the decomposition of hydrogen peroxide to water and oxygen. Catalase activity was assayed in tissues by measuring the rate of hydrogen peroxide (H₂O₂) decomposition according to the method described by Aebi ³⁰. Briefly, 250 μl of homogenate (100 mg of tissue in 0.9 ml KCl), 250 μl H₂O₂ (30 mmol in phosphate-buffered saline 50 mmol/l), and 250 μl of phosphate-buffered saline were added. The contents were shaken and incubated for 5 minutes, and then titanium sulfate (TiOSO₄) was added. The absorbance was measured at 240 nm.

Statistical Analysis: All data are presented as means ± SEM of 6 rats per group. Statistical analysis was carried out by STATISTICA (version 4.1; Statsoft, Tulska, Okla). The significance of differences was performed with one-way analysis of variance at a significance level of P<0.05. Further specific group differences were determined with Tukey honestly test.

RESULTS:

Phytochemical Screening and Antioxidant Activity: *Z. gaetulum* total polyphenols content and antioxidant activity were found to be higher **Table 1**. The highest antioxidant potential may be related to phenolics and flavonoids content of this extract.

TABLE 1: CONCENTRATION OF THE MAJOR CONSTITUENTS AND ANTIOXIDANT ACTIVITY OF THE LYOPHILIZED AQUEOUS EXTRACT OF *Z. GAETULUM*

Total polyphenols ^a	Flavonoids ^b	Tannins ^a	DPPH scavenging IC ₅₀ ^c	FRAP ^a
90.94 ± 12.73	16.98 ± 2.46	28.38 ± 12.41	25.84 ± 6.44	29.60 ± 3.78

Values are mean ± SEM of 6 tests. ^aexpressed as gallic acid equivalent (GAE) in mg/100 g dw; ^bexpressed as Quercetin equivalent (QE) in mg/100 g dw; ^cAntioxidant activity expressed as μg/ml

Effect of *Z. gaetulum* on Diabetic Rats in Acute Study: Induction of diabetes in the experimental rats was confirmed by higher fasting blood glucose level. A significant ($p<0.05$) increase in the level of blood glucose and glycosylated hemoglobin was observed in diabetic rat (untreated group) when compared to the control group. Administration of the *Zg* extract to diabetic rats showed reduction in blood glucose levels (-58%) and induced a significant decrease of glycosylated hemoglobin levels **Table 2**.

However, serum insulin concentrations were

similar in both groups. HOMA-IR value was reduced by 57%. Inversely, the QUICKI index was increased (+10%). In the diabetic rats, daily administration of *Zg* extracts induced a significant increase of plasma albumin levels (+24%) **Table 2**. Inversely, uric acid level and aspartate aminotransferase (AST) activity were decreased by 24% and 30%, respectively. However, there were no significant changes of alanine aminotransferase (ALT) activity.

Effect of *Z. gaetulum* on Serum Lipid Concentrations and Atherogenicity Ratios: The

diabetic animals showed several lipid abnormalities. *Zygodhymallium gaetulum* treatment lowered significantly serum TC (-45%), LDL-HDL₁-C (-57%) and VLDL-TG (-75%), whereas HDL-C concentrations were similar in both groups

Table 3. Serum TG and PL levels were increased by 55% and 57%, respectively, in DZg vs D group. TC/HDL-C and LDL-HDL₁-C/HDL-C ratios were respectively, 2.1- and 2.5-fold lower, in Zg-treated than untreated diabetic group.

TABLE 2: BIOCHEMICAL PARAMETERS IN CONTROL, UNTREATED DIABETIC, AND Zg-TREATED DIABETIC RATS

	Diabetic		
	Control	Untreated	Zg-treated
Glycemia (mmol/l)	6.16 ± 0.35	27.09 ± 2.29 [‡]	11.28 ± 2.41*
HbA1c (%)	2.17 ± 1.72	10.11 ± 0.38 [‡]	7.29 ± 1.90*
Insulin (pmol/l)	82.41 ± 1.28	64.13 ± 4.10	67.31 ± 5.83
QUICKI	0.32 ± 0.003	0.27 ± 0.004 [‡]	0.30 ± 0.006*
HOMA-IR	3.23 ± 0.25	11.15 ± 1.62 [‡]	4.81 ± 0.67*
HOMA-B	38.79 ± 7.61	6.82 ± 0.27 [‡]	17.82 ± 4.56
Albumin (g/l)	37.22 ± 2.94	20.77 ± 1.06	29.47 ± 0.35*
Uric acid (mmol/l)	295.51 ± 1.06	376.12 ± 0.59	286.87 ± 0.36*
AST (IU/l)	51.23 ± 7.27	91.43 ± 3.09	63.77 ± 3.71*
ALT (IU/l)	50.82 ± 3.24	60.01 ± 2.47	54.25 ± 2.47

Values are mean ± SEM of 6 rats per group. [‡]P<0.05 vs. control rats; *P<0.05 vs. diabetic rats

TABLE 3: SERUM LIPID CONCENTRATIONS AND ATHEROGENICITY RATIOS IN CONTROL, UNTREATED AND Zg-TREATED DIABETIC RATS

	Diabetic		
	Control	Untreated	Zg-treated
TC (mmol/l)	1.21 ± 0.1	2.01 ± 0.21 [‡]	1.10 ± 0.20*
LDL-HDL ₁ -C (mmol/l)	0.41 ± 0.08	0.73 ± 0.04	0.31 ± 0.05*
HDL-C (mmol/l)	0.64 ± 0.06	0.43 ± 0.01 [‡]	0.45 ± 0.06
TG (mmol/l)	0.33 ± 0.07	0.96 ± 0.02 [‡]	0.42 ± 0.04*
VLDL-TG (mmol/l)	0.085 ± 0.003	0.34 ± 0.01 [‡]	0.08 ± 0.01*
PL (mmol/l)	0.47 ± 0.33	1.31 ± 0.07 [‡]	0.55 ± 0.06*
TC/HDL-C	1.81 ± 0.88	4.74 ± 0.09 [‡]	2.22 ± 0.36*
LDL-HDL ₁ -C/ HDL-C	0.64 ± 0.05	1.71 ± 0.14 [‡]	0.69 ± 0.01*

Values are mean ± SEM of 6 rats per group. [‡]P<0.05 vs. control rats; *P<0.05 vs. diabetic rats

Effect of *Z. gaetulum* on Serum apo A-I levels, LCAT, and PON1 Activities: In the untreated diabetic group compared with the control, LCAT and PON1 activities levels were decreased by -61% and -52%, respectively. However, compared with the untreated diabetic group, the Zg aqueous extract supplementation showed increased LCAT and PON1 activities (+43%). In addition, serum apo A-I concentrations tended to be higher, but not significantly **Table 4.**

TABLE 4: APO A-1 (g/l), LECITHIN: CHOLESTEROL ACYLTRANSFERASE (nmol/ml/h) AND PARAOXONASE ACTIVITIES (IU/l).

	Diabetic		
	Control	Untreated	Zg-treated
Apo A-1	2.76±0.41	1.19±0.80	2.02±0.31
LCAT	19.11±1.38	11.60±3.17 [‡]	18.85±3.95*
PON1	9.05±0.70	4.30±0.62 [‡]	7.52±0.70*

Values are mean ± SEM of 6 rats per group. [‡]P<0.05 vs. control rats; *P<0.05 vs. diabetic rats

Effect of *Z. gaetulum* on Liver and Kidney Lipid Peroxidation: Thiobarbituric acid reactive substances analysis was conducted to estimate the lipid oxidation development. In the diabetic group in comparison with the control, TBARS values were increased significantly (P<0.05). Inversely, Zg-treatment normalized TBARS levels and induced a significant decrease in the liver (-46%) and kidney (-40%) **Fig. 1.**

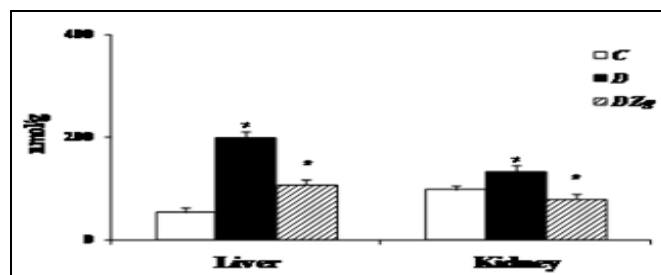


FIG. 1: TBARS LEVELS IN LIVER AND KIDNEY

Values are mean ± SEM of 6 rats per group. [‡]P<0.05 vs. control rats; *P<0.05 vs. diabetic rats

Antioxidant Enzyme Activities: In untreated diabetic compared with the control rats, the antioxidant enzyme activities in liver and kidney declined significantly ($P < 0.05$) **Fig. 2**. In the liver, SOD and GSSH-Red activities levels were decreased by -45% and -47%, respectively. Inversely, CAT activity was increased (+44%). However, SOD, GSH-Px and CAT activities were lower (-54%, -76% and -49%), respectively.

In *Zg*-treated vs. untreated diabetic rats, SOD was increased in the liver (+28%), But this activity remained unchanged in the kidney **Fig. 2**. Furthermore, GSH-Px activities were increased respectively by +76% and +58%, respectively. However, GSSH-Red activity was higher (+41%), only in the kidney. CAT activity was increased in the kidney (+35%), but the liver was not sensitive.

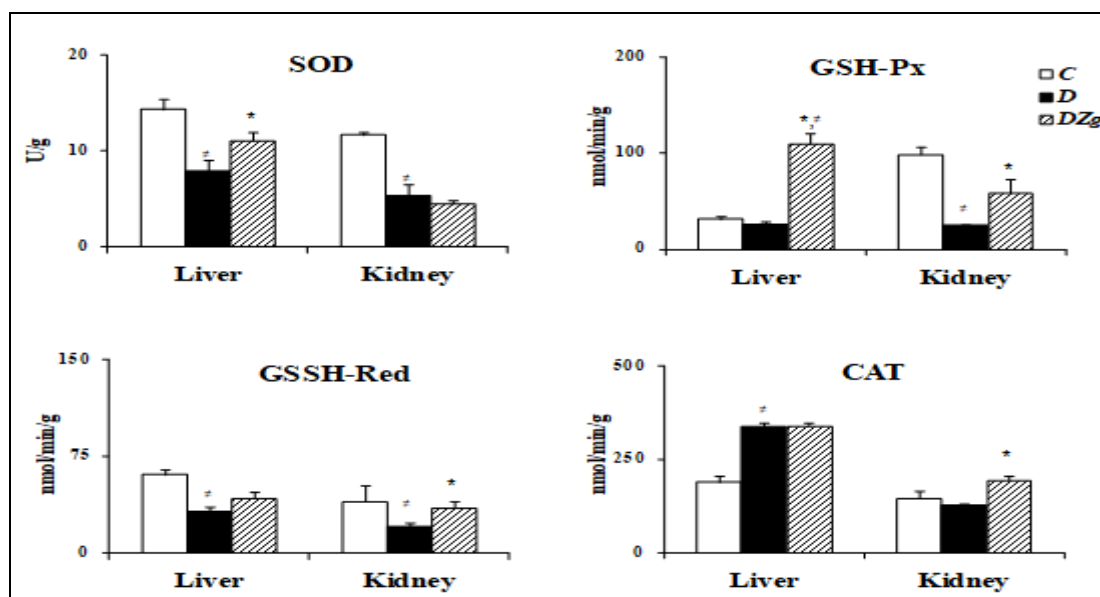


FIG. 2: ANTIOXIDANT ENZYME ACTIVITIES IN LIVER AND KIDNEY

Values are means \pm SEM of 6 rats per group. $^{\#}P < 0.05$ vs. control rats; $*P < 0.05$ vs. diabetic rats

DISCUSSION: Herbs are used in various domains, including medicine, nutrition, and cosmetics. Numerous species have been recognized to have medicinal properties. In our study, experimental evaluation of the antihyperglycemic potential of *Z. gaetulum* has shown that administration of the extract to diabetic rats reduced fasting blood glucose, prevents lipid peroxidation by enhancing PON1, LCAT activities and ameliorates the antioxidant enzymes activity, especially in liver and kidney. Diabetes mellitus causes a disturbance in the uptake of glucose as well as glucose metabolism. In contrast, the increased levels of plasma glucose in diabetic rats were lowered by *Z. gaetulum* administration. The glucose-lowering activity of *Zg* may be related to both Pancreatic (enhancement of insulin secretion) and extrapancreatic (peripheral utilization of glucose) mechanism. Some studies suggest that the antihyperglycemic action of traditional antidiabetic plant extracts may be due in part to decreased glucose absorption³¹.

The increase in the level of glycosylated hemoglobin (HbA1c) in the diabetic group of rats is due to the presence of large amounts of blood glucose which reacts with hemoglobin to form glycosylated hemoglobin³². The level of HbA1c was decreased after the administration of *Z. gaetulum*. This result was concomitant with the hypoglycemic effect induced by *Zg* extract. Generally, phenolic compounds such as flavonoids, phenolic acid and tannins are considered to be a major contributor to the antioxidant activity in medicinal plants.

This can explain the important antioxidant potential of the *Z. gaetulum* extract. On the other hand, the increased acid uric level was noted in the diabetic group. Interestingly, the higher levels of uric acid in diabetic rats are diminished significantly after the administration of *Z. gaetulum*. ALT and AST are common intracellular enzymes that increase liver damage induced by diabetes³³.

Our data showed that plasma alanin aminotransferase (ALT) and aminotransferase (AST) activities were significantly increased in diabetic rats. In contrast, they were diminished in Zg-treated group, most likely due to its antioxidant properties.

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease³⁴. Reduction in serum total cholesterol and triglycerides through dietary *Z. gaetulum* therapy has been noted. Thus, this plant extracts ameliorated diabetes-induced dyslipidemia by reducing the levels of cholesterol in LDL-HDL₁ and TG in VLDL. It could be suggested that these effects were explained by the presence of flavonoids in Zg extract. These compounds have an antioxidant effect and are effective to reduce cholesterolemia, inhibit thrombosis and to dilate the coronary arteries³⁵.

The previous report of Sinha *and al*³⁶ suggests that the hypolipidemic activity may be attributed to inhibition of oxidative stress. Recently, it has been reported that the oxidation of glucose in diabetes mellitus is the major cause for generating oxidative stress³⁷. In addition, our result showed that TBARS levels in the liver and kidney were decreased significantly in Zg-treated diabetic rats compared to untreated diabetic rats. These results indicated that Zg extract might protect the tissues against the cytotoxic action and oxidative stress of streptozotocin. Moreover, the reduction in the lipid peroxidation could be due to the improvement of the glycemic control and the increased of antioxidant status, since Zg aqueous extract was highly antioxidant and had hypoglycemic activity in STZ-diabetic rats.

Elevated free radical concentration and lipid peroxidation decrease the antioxidant defense in the biological systems³⁸. The present data indicate that *Z. gaetulum* induced increased significantly SOD, CAT, GSH-Px, GSSH-Red activities content in liver and kidney (P<0.05), indicating the efficacy of the Zg extract to reduce oxidative stress *in vivo*. The constituents of *Z. gaetulum* such as flavonoids and ascorbic acid have antioxidant activity, so the observed antioxidant and antidiabetic activities of this extract may be attributed to the presence of these bioactive principles and their synergistic

properties. Several studies also reported that LCAT and paraoxonase activities lowered in diabetic patients³⁹. In the current study, *Z. gaetulum* increased LCAT and PON1 activities and holds the potential role in the management of dyslipidemia, by virtue of its antioxidant property.

CONCLUSION: The result of our study showed that lyophilized aqueous extract of *Z. gaetulum* possesses antihyperglycemic and hypolipidemic activities in STZ induced diabetic rats; these effects may be due to the antioxidant potential of this plant. Moreover, Zg extract prevents lipid peroxidation by enhancing PON1 and LCAT activities and ameliorates the antioxidant status of the liver and kidney, thereby protecting diabetic dyslipidemia. The study is further extended to identify and characterize the exact active phytoconstituents which are responsible for the observed effects.

ACKNOWLEDGEMENT: This research was supported by the Algerian Ministry of Higher Education and Scientific Research

COMPETING INTEREST: All authors of this research have no conflict of interest related to employment, consultancies, stock ownership, grants or other funding

REFERENCES:

1. Shen X: Oxidative stress and diabetic cardiovascular disorders: roles of mitochondria and NADPH oxidase. *Can. J of Physiol. Pharm* 2010; 88: 241-48.
2. Mackness B, Durrington PN, Boulton AJ, Hine D and Mackness MI: Serum paraoxonase activity in patients with type 1 diabetes compared to healthy controls. *Eur J Clin Invest* 2002; 32: 259-64.
3. Guan JZ, Tamasawa N and Murakami H: HMG CoA reductase inhibitor, simvastatin improves reverse cholesterol transport in type 2 diabetic patients with hyperlipidemia. *J Ather Thromb* 2008; 15: 20-25.
4. Jonas A: Lecithin cholesterol acyltransferase. *Biochim Biophys Acta* 2000; 1529: 245-46.
5. Rousset X, Vaisman B, Amar M, Sethi AA and Remaley AT: Lecithin: cholesterol acyltransferase from biochemistry to role in cardiovascular disease. *Curr. Opin. Endocrinol Diabetes Obes* 2009; 16: 163-71.
6. Vekic J, Kotur-Stevuljevic J, Jelic-Ivanovic Z, Spasic S, Spasojevic-Kalimanovska V and Topic A: Association of oxidative stress and PON1 with LDL and HDL particle size in middle-aged subjects. *Eur J Clin Inv* 2007; 37: 715-23.
7. Itamar R, Jay SS and Eleazar S: Diabetes: From research to diagnosis and treatment. *Martin Dunitz UK* 2003; 8: 139.
8. Nobecourt E, Davies MJ and Brown BE: The impact of glycation on apolipoprotein A-I structure and its ability to

- activate lecithin: cholesterol acyltransferase. *Diabetologia* 2007; 50: 643-53.
9. Isik A, Koca SS, Ustundag B, Celik H and Yildirim A: Paraoxonase and arylesterase levels in rheumatoid arthritis. *Clin Rheumatol* 2007; 26: 342-48.
 10. Guo H, Ling W, Wang Q, Liu C, Hu Y and Xia M: Cyanidin 3-glucoside protects 3T3-L1 adipocytes against H₂O₂- or TNF- α -induced insulin resistance by inhibiting c-Jun NH2-terminal kinase activation. *Biochem pharmacol* 2008; 75: 1393-01.
 11. Jaouhari JT, Lazrek HB, Seddik A and Jana M: Hypoglycaemic response to *Zygophyllum gaetulum* extracts in patients with non-insulin-dependent diabetes mellitus. *Journal of Ethnopharmacology* 1999; 64: 211-17.
 12. Ait El, Cadi M, Makram S, Ansar M, Khabbal Y, Alaoui K, Faouzi MA, Cherrah Y and Taoufik J: Activité anti-inflammatoire des extraits aqueux et éthanologique de *Zygophyllum gaetulum*. *Annales Pharmaceutiques Françaises* 2011; 1-28.
 13. El Hamsas El Youbi A, Boustab D, Ouahidi I and Aarab L: Criblage pharmacologique primaire d'une plante endémique originaire du Sud Marocain (*Tetraena gaetula*). *Comptes Rendus Biologies* 2010; 333: 736-43.
 14. Skim F, Lazrek BH, El Amri H, Kaaya A and Jana M: Toxicological studies on *Globularia alypum* and *Zygophyllum gaetulum* in rats. *Phytotherapy Research* 1998; 12: 592-94.
 15. Wong SP, Leong LP and Koh JHW: Antioxidant activities of aqueous extracts of selected plants. *Food Chem* 2006; 99: 775-83.
 16. Djeridane A, Yous M, Nadjemi B, Boutassouna D, Stocker P and Vidal N: Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem* 2006; 97: 654-60.
 17. Hagerman AE and Butler LG: Protein precipitation method for the quantitative determination of tannins. *J Agric Food Chem* 1978; 26: 809-12.
 18. Hua Li, Xiaoyu Wang Peihong Li, Yong Li and Hua Wang: Comparative Study of Antioxidant Activity of Grape (*Vitis vinifera*) Seed Powder Assessed by Different Methods. *J of Food and Drug Analysis* 2008; 16: 67-73.
 19. Yen GC and Duh PD: Scavenging effect of methanolic extracts of Peanut hulls on free-radical and active-oxygen species. *J Agri Food Chem* 1994; 42: 629-32.
 20. Katalinic V, Modun D, Music I and Boban M: Gender differences in antioxidant capacity of rat tissues determined by 2, 2'-azinobis (3-ethylbenzothiazoline 6-sulfonate; ABTS) and ferric reducing antioxidant power (FRAP) assays. *Comp Biochem Physiol C Toxicol Pharmacol* 2005; 140: 47-52.
 21. Council of European Communities: Council instructions about the protection of living animals used in scientific investigations. Official J. L358 of 18-12-1986, Corrigendum Official J. L117 1987; 05-05.
 22. Burstein M, Scholnick HR and Morfin R: Rapid method for isolation of lipoproteins from human serum by precipitation with polyanions. *J Lip Res* 1970; 11: 583-95.
 23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28(7): 412-9.
 24. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G and Quon MJ: Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *JCEM* 2000; 85: 2402-10.
 25. Glomset JA and Wright JL: Some properties of cholesterol esterifying enzyme in human plasma. *Biochem Biophys Acta* 1964; 89: 266-71.
 26. Knipping G: Isolation and properties of porcine lecithin: cholesterol acyltransferase. *Eur J Bioc* 1986; 154: 289-94.
 27. Schiavon R, De Fanti E, Giavarina D, Biasioli S, Cavalcanti G and Guidi G: Serum paraoxonase activity is decreased in uremic patients. *Clin Chim Acta* 1996; 247: 71-80.
 28. Ohkawa H, Ohishi N and Yagi K: Assay for lipid peroxidases in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 85: 351-58.
 29. Goldberg DM and Spooner RJ: Glutathione reductase. In: *Methods of enzymatic analysis*. 2nd ed. Germany: Berg Meyer H ed, Verlag Chemie, Weinheim 1992: 258-65.
 30. Aebi H: Catalase. In: Berg Meyer, H. (Ed.), *methods of enzymatic analysis*, 2nd ed. Germany: Verlag Chemie, Weinheim; 1974: 673-84.
 31. Gallagher AM, Flatt PR, Duffy G and Abdel-Wahab YHA: The effects of traditional antidiabetic plants on *in-vitro* glucose diffusion. *Nutr Res* 2003; 23: 413-24.
 32. Can A, Akev N, Ozsoy N, Bolkent S, Arda BP, Yanardag R and Okyar A. Effect of Aloe vera leaf gel and pulp extracts on the liver in type-II diabetic rat models. *Biol Pharm Bull* 2004; 27: 694-98.
 33. Davidson MB: *Diabetes Mellitus: Diagnosis and Treatment*. Wiley. Hoboken. NJ 1981; 109.
 34. Agrawal RP, Sharma P, Pal M, Kochar A and Kochar DK: Magnitude of dyslipidemia and its association with micro and macrovascular complications in type 2 diabetes: A hospital-based study from Bikaner (Northwest India). *Diabetes Res Clin Pract* 2006; 73: 211-14.
 35. Shao B, Guo HZ and Cui YJ: Simultaneous determination of six major stilbenes and flavonoids in *Smilax china* by high-performance liquid chromatography. *Journal Pharmaceut Biomed* 2007; 44: 73742.
 36. Sinha M, Manna P and Sil PC: Terminalia arjuna protects mouse hearts against sodium fluoride-induced oxidative stress. *J Med Food* 2008; 11: 73340.
 37. Yasuhiro H, Hideki F and Masafumi F: Role of oxidative stress in diabetic bone disorder. *Bone* 2009; 45: 35-38.
 38. Halliwell B: Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and *in-vivo* studies? *Arch Biochem Biophys* 2008; 476: 107-12.
 39. Dirican M, Serdar Z, Sarandöl E and Sürmen-Gür E: Lecithin: cholesterol acyltransferase activity and cholesteryl ester transfer rate in patients with diabetes mellitus. *Turk J Med Sci* 2003; 33: 95-01.

How to cite this article:

Guenzet A, Krouf D, Zennaki S and Berzou S: *Zygophyllum gaetulum* aqueous extract protects against diabetic dyslipidemia and attenuates liver and kidney oxidative damage in streptozotocin induced-diabetic rats. *Int J Pharm Sci & Res* 2014; 5(11): 4709-17. doi: 10.13040/IJPSR.0975-8232.5(11).4709-17.