DOI:10.5138/09750185.2451

AR JOURNALS ADVANCED RESEARCH

International Journal of Phytomedicine
2020;12(4):086-096

RESEARCH ARTICLE

Zygophyllum album aqueous extract reduces oxidative damage in red blood cells and attenuate pro-inflammatory plasma markers in hypercholesterolemic-diabetic rats

Yasmina BAHLIL, Djamil KROUF* and Nawal TALEB-DIDA

Abstract

Zygophyllum album (*Z. album*) is used in traditional medicine for a long time for its anti-diabetic activities. Thisstudy had to assess Z. album extract supplementation effects on redox and inflammatory statuses in hypercholesterolemic-diabetic rats. Male rats Wistar (n=36), weighing 200±10 g were divided into three groups (n=12). The first group was rendered hypercholesterolemic (HC) with a high cholesterol diet (1 %). The second group was rendered diabetic (D) by intraperitoneal injection of streptozotocin (STZ) (35 mg/kg of body weight). The third group was rendered hypercholesterolemic-diabetic (HC-D); each group was divided into two groups (n=6), untreated groups (HC, D, HC-D) and treated groups with 1 % *Z. album* extract (HC-Za, D-Za and HC-D-Za). After 28 days, Z. album treatment lead to a decrease in RBC's TBARS contents in HC-Za (-44 %), D-Za (-66 %) and HC-D-Za (-23 %) groups. An increase in the antioxidant enzymes activities (superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase) was observed in HC-Za, D-Za and HC-D-Za ($p < 0.05$). IL-1 β and IL-6 concentrations were reduced by -44, -50 and -33 % and -49, 38 and -41 %, respectively in treated groups. A decrease in TNF-α as well as homocysteine and CRP was observed in *Z. album* treated groups ($p < 0,05$). In conclusion, *Z. album* reduces radical attack and improves the anti-inflammatory proprieties in hypercholesterolemic-diabetic rats.

Keywords: Hypercholesterolemia; Diabetes; Zygophyllum album; RBC; Oxidative stress; inflammation

Introduction

Cardiovascular diseases (CVD) are considered the leading cause of morbi-mortality worldwide and refer to various medical conditions [1]. Various risk factors predisposing to CVD, such as hypercholesterolemia, diabetes, obesity, high blood pressure and aging, lead to vascular dysfunction and CVD, partly due to oxidative and inflammatory stress [2, 3].

Reactive oxygen species (ROS) and other oxidative stress factors are associated with cardiovascular and inflammatory diseases [4, 5]. ROS play a key role in the inflammatory component of CVD, where they induce the formation of inflammasomes, which in turn support the secretion and transformation of pro-inflammatory cytokines such as interleukin IL-1 β and IL-8 by activation of caspase-1 [3, 6]. In addition, recent research has shown that ROS have the power to trigger secretion of pro-

*Correspondence: lncmkrouf@gmail.com

Department of Biology, Faculty of Natural and Life Sciences, Université Oran, 1 Ahmed Ben Bella, Oran, Algeria. Fax: 0021351074435

Full list of author information is available at the end of the article.

Received: 02 Oct 2020, Accepted:25 Nov 2020

inflammatory cytokines, and they can also induce the production of ROS [1]. Thus, several studies have reported that oxidative stress plays a central role in mediating the production and secretion of pro-inflammatory cytokines, which can also be triggered by mitochondrial ROS [7, 8], connecting ROS with inflammation [1]. Various chronic conditions associated with inflammation, including *diabetes mellitus* and CVD, are characterized by excessive production of ROS [1, 9].

Furthermore, oxidative stress is a major feature of CVD, although a causal link has not, to date, been established by large-scale clinical trials. However, there is a strong link between oxidative stress, inflammation and the onset and progression of CVD, which may be the missing link between oxidative stress and cardiovascular mortality [10].

Several authors have shown that overproduction of ROS is causally related to diabetes and diabetic complications [11, 12]. Also, a large number of studies suggest that oxidative stress plays an important role in the pathogenesis of diabetes [13]. In diabetes, oxidative stress causes glucotoxicity and lipotoxicity of β cells, resulting in increased β cell destruction by a primary process of diabetes: hyperglycemia and hyperlipidemia. In vitro and in vivo studies have suggested that high concentrations of glucose and lipids are indeed harmful to β cells [14]. On the other hand, inflammation plays a causal and potentially crucial role in the development and installation of diabetes [15, 16]. In addition, high concentrations of inflammation biomarkers such as fibrinogen, C-reactive protein (CRP) and IL-6 have been associated with the atherogenic lipid profile of diabetes [17].

Furthermore, vascular dysfunction and CVD risk factors such as high blood pressure, hypercholesterolemia and coronary artery diseases are clearly related to inflammatory processes [10]. Oxidative stress and inflammatory processes involve several biochemical intermediates in the development of hypercholesterolemia and atherosclerosis. Increased ROS production may play a key role in chronic inflammatory responses [18]. The increase in cholesterol levels in arterial lumen is found to be involved in the increase of inflammatory lesions through the formation of pro-oxidant mediators in arterial wall cells and the development of atherosclerosis [19]. Excessive production of ROS supports convert LDL-C (low-density lipoprotein cholesterol) to LDL-C-Ox (LDL oxidized), recognized by macrophages. LDL-C-Ox-activated macrophages induce increased oxidative stress, which in turn contributes to the inflammatory response by secreting pro-inflammatory cytokines [20].

Throughout the world, traditional medicine is either the primary mode of health care delivery or a complement to it. This medicine is an important and often underestimated part of health care. It exists in almost every country in the world and the request for services in this area is increasing.

For millions of people, herbal medicines are the main, if not the only, source of health care. These cares are easy to access and inexpensive. They are also culturally acceptable and many people believe in their therapeutic virtues [21]. The use of plants for their medicinal properties is a very olden practice. It takes its origins in the oldest civilizations and has been well preserved for centuries throughout the world [22]. The World Health Organization has promoted the use of alternative therapies integrated with medicinal plants since 1976. About 80% of the world's population uses medicinal plants for therapeutic purposes [23]. Herbal medicine, in Algeria, is an integral part of the local culture, the population has an important indigenous knowledge, acquired empirically through the generations. Many patients use medicinal plants as a treatment for many serious conditions and diseases, such as diabetes, high blood pressure and dyslipidemia, for several reasons: historical, cultural and economic [22, 24].

For a very long time, diabetes has been treated with medicinal plants based on information from traditional medicine. There are several medicinal plants species, popularly used in the treatment of *diabetes mellitus*. Ethnopharmacological surveys indicate that more than 1200 plants are used worldwide, in traditional medicine, for their hypothetical hypoglycemic activity [25, 26].

According to Azzi et al., [24] out of 470 diabetics, 28% patients use only medicinal plants as a treatment, or in combination with a conventional treatment for diabetes such as metformin. On the other hand, different parts of several plants have medicinal properties such as ginger, nuts, pumpkin, red fruits and yellow onion that have a beneficial effect on hyperlipemia by reducing plasma levels of total lipids, triglycerides and cholesterol [27].

However, few studies take interest to *diabetes mellitus* associated to hypercholesterolemia; in addition, there is no research on the effects of aqueous extract from the *Zygophyllum album* (Z. album) on these two associated cardiovascular risk factors: hypercholesterolemia and diabetes.

For its anti-diabetic power, *Zygophyllum album* known as its vernacular name "Aggaya" has long been used by natives who consume mainly its leaves in infusion [24]. Antibacterial and antioxidant activities of Z. album leaves were also noted [28]. In addition, El Ghoul et al., [29] reported a vasorelaxant and anti-hyperglycemic effect of the aqueous extract of *Z. album* leaves in mice rendered diabetic by streptozotocin injection (STZ).

In this study, our aim is to evaluate the effects of *Z. album* lyophilized aqueous extract supplementation (1% in diet) in the

redox and inflammatory status in hypercholesterolemic-diabetic rats.

Materials & Methods

Album lyophilized aqueous extract preparation

Preparation of the lyophilized aqueous extract of *Z. album* leaves was realized from plant material, as previously described [30]. The crude yield of the lyophilized extract was approximately 30 % (wt/wt). It was stored at ambient temperature until further use.

Animals and dietary treatments

The experiments were approved by the Ethical Committee of the Faculty of Natural and Life Sciences, University of Oran 1 Ahmed Benbella (Algeria). These guidelines were in accordance with the European Council Legislation 86/609/EEC for the protection of experimental animals [31].

In total, 36 male Wistar rats (Iffa Credo, l'Arbresle, Lyon, France) weighing 200±10 g were fed an upkeep diet (ONAB) containing 18 % protein and were housed in stainless steel cages under standard environmental conditions (23±1° C, 55±5 % humidity and 12-hour light cycle (day/night).

Hypercholesterolemia i nduction

Among the 36 rats, 24 were rendered hypercholesterolemic by consuming a standard diet containing 20 % casein enriched with 1 % dietary cholesterol and 0.5 % cholic acid (Sigma-Aldrich Chemie, Germany). To check hypercholesterolemia installation fixed in rats from 4.92±0.91 mmol/L [32], blood samples were taken from the caudal vein. The collected blood was centrifuged at 1000 x g for 20 minutes at 4° C (Sigma, 4K10 Bioblock Scientific, Germany) then total cholesterol was measured on the serum according to a color enzyme method. After 5 weeks of high cholesterol-enriched diet consumption, rats became hypercholesterolemic (HC) with an average total cholesterol about 5.25±0.43 mmol/L compared to the obtained value at the experiment time zero, which was < 3.90 mmol/L.

Diabetes i nduction

After a fasting night, hyperglycemia was induced in normoglycemic rats (5.25±0.43 mmol/L) (n=12) by intraperitoneal injection of a single dose of streptozotocin (STZ) (Sigma, St Louis, USA) at a dose of 35 mg/kg of body weight. STZ was dissolved in freshly prepared citrate buffer (0.05 M, pH=4.5) [33]. Hyperglycemia was confirmed 48 hours after STZ injection, on a blood sample taken from caudal vein using a glucometer (One Call Extra, ACON Laboratories, San Diego, USA). The 12 rats rendered diabetic (D) had hyperglycemia ≥ 7.82 mmol/L [34].

Diabetes induction in hypercholesterolemic animals

Rats (n=12) with previously installed hypercholesterolemia (HC) were then rendered diabetic (HC-D) by intraoperative injection of a single dose of STZ under the same conditions described above.

Zygophyllum album lyophilized aqueous extract treatment

Experimental animals were divided into six groups of 6 rats each: three untreated groups consumed a standard enriched diet with 1 % cholesterol (HC and HC-D) or not (D) and three treated groups consumed the same diet supplemented with 1 g/kg of Zygophyllum album lyophilized aqueous extract enriched with 1 % cholesterol (HC-Za, HC-D-Za) or not (D-Za), for 28 days **(Table 1)**. Rats received water and food adlibitum renewed once a day.

Blood samples

After 4 weeks of the experimentation, animals were fasted overnight and anesthetized with chloral hydrate 10 % (3 mL/kg body weight) and euthanized with an overdose. Blood was obtained from abdominal aorta of rats and collected into tubes containing ethylene-diaminetetra acetic acid-Na2 (Sigma, St Louis, Mo). Blood plasma was prepared by low speed centrifugation at 1000 g for 20 min at 4 °C.

The plasma supernatant was removed for inflammation markers assessments. While blood pellet containing the red blood cells (RBC) was washed and then treated differently, depending on the specificity of each biochemical assay carried out later.

Red blood cells (RBC) washing and obtaining

The blood pellet was washed and then centrifuged at 4000 g for 10 min at 4 °C in PBS buffer (Na_2HPO_4: 1,44 g/L, NaCl: 8 g/L, KCl: 0,2 g/L, distilled water qsp 50 mL), (pH=7,4). The process was repeated 3 times until the supernatant will be clear. After supernatant removal, the resulting pellet containing the RBC was conserved for the several assay below.

Rats were randomly divided into three groups (n=12). The first group was rendered hypercholesterolemic with an enriched cholesterol diet (1 %) (HC). The second group was rendered diabetic by intraperitoneal injection of streptozotocin (STZ) (35 mg/kg of body weight) (DM). The third group was hypercholesterolemic and diabetic (HC-D); each group was divided into two groups (n=6), untreated groups (HC, D, HC-D) and treated groups with 1 % Z. album extract (HC-Za, D-Za and HC-D-Za) for 4 weeks.

Table 1 Composition of the experimental diet(g/100g diet[a])

Ingredients	HC	D	HC-D	HC- Za	D- Za	HC-D- Za
Casein [b]	20	20	20	20	20	20
Cornstarch [c]	59.5	61	59.5	58.5	60	58.5
Sucrose [d]	4	4	4	4	4	4
Sunflower oil [e]	5	5	5	5	5	5
Cellulose [b]	5	5	5	5	5	5
Mineral mix [f]	4	4	4	4	4	4
Vitamin mix [g]	1	1	1	1	1	1
Cholesterol [b]	1	-	1	1	-	1
Cholic acid [b]	0.5	-	0.5	0.5	-	0.5
Z. album lyophilized extract [h]	-	-	-	1	1	1

[a] Diets were isocaloric (17780 J/kg diet) and given in powdered form.
[b] Sigma-Aldrich Chemie.
[c] ONAB, Sidi Bel Abbès, Algeria.
[d] ENASUCRE, Sfisef, Algeria.
[e] CEVITAL SPA, BéjaÑÛa, Algeria.
[f] UAR 205B (Villemoisson, 1360, Epinay/S/Orge, France). Mineral mixture provided the following amounts (mg/kg diet): $CaHPO_4$, 17 200; KCl, 4000; NaCl, 4000; MgO_2, 420; $MgSO_4$, 2000; Fe_2O_3, 120; $FeSO_4$,$7H_2O$, 200; $MnSO_4$, H_2SO_4, H_2O, 98; $CuSO_4$, $5H_2O$, 20; $ZnSO_4$, 80; $CuSO_4$, 80; $CuSO_4$, $7H_2O$; and KI, 0,32.
[g] UAR 200 (Villemoisson, 91360). Vitamin mixture provided the following amounts (mg/kg diet): vitamin A, 39 600 UI; vitamin D_3, 5000UI; vitamin B1, 40; vitamin B_2, 30; vitamin B_3, 140; vitamin B_6, 20;vitamin B_7, 300; vitamin B_{12}, 0.1; vitamin C, 1600; vitamin E, 340; vitamin K, 3.80; vitamin PP, 200; choline, 2720; folic acid, 10; paraaminobenzoic acid, 180; biotin, 0.6; and cellulose, qsp, 20 g;
[h] Zygophyllum album (Z. album), prepared in our laboratory as previously described.

RBCs lipid peroxidation assay

As a marker of lipid peroxidation, thiobarbituric acid reactive substances (TBARS) of RBC were measured according to Quintanilha method [35] using malondialdehyde (Sigma–Aldrich, l'Isle d'Abeau, France) as a standard. 100 μL of freshly washed RBC, were diluted in 900 μL of PBS tampon containing 2 mmol/L of sodium azide then incubated in the presence of 100 μL of H_2O_2 (1.15%), for 1 hour to 37 °C [36]. This reaction was inhibited with 1 mL of 20% trichloroacetic acid (TCA). After centrifuging to 4000 x g for 10 minutes, the supernatant corresponding to the RBC lysate was collected. 100 μL of BHT (2% in ethanol) (Sigma-Aldrich Chemie, Germany) and 1 mL (TBA) (Sigma-Aldrich Chemie, Germany) (TBA 0.37% in HCL at 0.5 N in final TCA concentration at 15%) were added to the supernatant. After incubation at 85 °C for 30 minutes and cooling in ice, the samples were centrifuged to 2000 x g for 10 min to 4 °C. After cooling and centrifugation, supernatant absorbance was measured at 535 nm.

Reduced glutathione (GSH) determination in RBC

GSH concentration was determined with the method of Sedlak and Lindsay [37] **,** based on color complex formation of non-protein sulfhydryl groups, which were separated by deproteinization with trichloroacetic acid (Carlo Erba, Rodano, Italy), with Ellmann reagent (5.5'-dithiobis-2 nitrobenzoic acid; Sigma, St Louis, USA). To 1 mL of freshly washed RBC were added 800 μL of iced distilled water and 200 μL of TCA (50%). The mixture was agitated for 10 minutes and centrifuged to 1200 x g for 15 minutes. After centrifugation, 400 μL of supernatant corresponding to RBC lysate were mixed with 800 μL of tris buffer (0.4 mol/L, pH=8.9) and 20 μL of DTNB (0.01 mol/L). After 5 minutes of incubation, the absorption was measured at 412 nm.

Antioxidant RBCs enzymes activities assay

To 2 mL of freshly washed RBC was added ice deionized water (1/4; V/V) then centrifuged to 10,000 x g for 15 minutes at 4 °C. The collected supernatant is the RBC lysate that will be used for antioxidant enzymes determinations.

Superoxide dismutase (SOD, EC. 1.15.1.1) was assayed using a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine (kit; Cayman).

Glutathione peroxidase assay measures (GSH-Px, EC. 1.11.1.9) activity indirectly by a coupled reaction with glutathione reductase (kit; Cayman).

Glutathione reductase (GSSH-Red, EC. 1.6.4.2) activity was estimated by measuring the rate of NADPH oxidation (kit; Cayman).

Catalase (CAT, EC. 1.11.1.6) method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2 (kit; Cayman).

Plasma inflammatory status evaluation

Tumor necrosis factor (TNF- α), interleukins (IL-1β and IL-6), C-reactive protein (CRP) and homocysteine determination

Plasma TNF-α, IL-1 β, IL-6 and CRP assessments was based on immuno-enzymatic techniques, Elisa (RayBio kit, Norcross GA, USA) based on the use of 2 antigenic-specific polyclonal antibodies present in rat plasma. The coloration was directly proportional to the concentration of the antigen in the sample or the standard. The reaction was stopped after the addition of H_2SO_4 and the absorption was measured at a wavelength of 450 nm.

Homocysteine was evaluated using the same technique cited above by kit method (LifeSpan's, Biosciences, Seattle, WA 98121, USA).

TNF- α, IL-1 β, IL-6, CRP and homocysteine plasma concentrations were determined using standard ranges based on mother solutions of TNF-α (500 pg/mL), IL-1 β (500 pg/mL), IL-6 (1000 pg/mL), CRP (200 mg/mL) and homocysteine (50 μmol/L).

Statistical analyses

All values were expressed as the means $\pm$ standard deviations (SDs) of six rats per group. Statistical analysis was carried out by STATISTICA (version 4.1; Statsoft, Tulska, Okla). The significance of differences in response to Z. album supplementation was analyzed by two-way analysis of variance followed by Tukey honestly significant difference test. Statistical significance was set at $p < 0.05$. Album effect: *CH-Za vs C; $\neq$DM-Za vs; $CH-DM-Za vs CH-DM

Results

TBARS and GSH RBCs assessment

Our results indicate a decrease in RBC's TBARS concentrations in HC-Za vs HC (-44%), D-Za vs D (-66%) and HC-D-Za vs HC-D (-23%), groups. In addition, GSH RBC's content was increased in the same groups, HC-Za vs HC (+37%), D-Za vs D (+33%) and HC-D-Za vs HC-D (+46%) **(Table 2)**.

RBC antioxidant enzymes activity

Compared to the untreated groups, an increase in SOD activity was noted in all Z. album treated groups, HC-Za, D-Za and HC-D-Za, this increase was by +34, +37 and +59%, respectively.

Similarly, GSH-Px activity was increased in HC-Za vs HC (+17%), D-Za vs D (+28%) and HC-D-Za vs HC-D (+20%) groups.

GSSH-Red activity was reduced in D-Za compared to D group (-28%), conversely, a significant activity was observed in HC-Za vs HC (+32%) and HC-D-Za vs HC-D (+15%) groups.

CAT activity was increased in all Z. album extract treated groups, by +29% in HC-Za, +38% in D-Za and +28% in HC-D-Za groups.

Rats were randomly divided into three groups (n=12). The first group was rendered hypercholesterolemic with an enriched cholesterol diet (1 %) (HC). The second group was rendered diabetic by intraperitoneal injection of streptozotocin (STZ) (35 mg/kg of body weight) (DM). The third group was hypercholesterolemic and diabetic (HC-D); each group was divided into two groups (n=6), untreated groups (HC, D, HC-D) and treated groups with 1 % Z. album extract (HC-Za, D-Za and HC-D-Za) for 4 weeks.

Note: Abbreviations: HC: Hypercholesterolemic rats group; HC-Za: Hypercholesterolemic rats group treated with Zygophyllum album (Z. album) aqueous extract (1%); D: Diabetic rats group; D-Za: Diabetic rats group treated with Z. album aqueous extract (1%); HC-D: Hypercholesterolemic-diabetic rats group; HC-D-Za: Hypercholesterolemic-diabetic rats group treated with Z. album aqueous extract (1%); TBARS : Thiobarbituric acid reactive species; GSH: Reduced glutathione; SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidise; GSSH-Red: Glutathione reductase; CAT: Catalase.

Inflammatory status assessment

Interleukin 1β (IL-1β) and Interleukin 6 (IL-6)

IL-1 β and IL-6 levels were decreased in Z. album treated groups (Table 2). In fact, IL-1 β concentrations were reduced by -44, -50 and -33%, respectively in HC-Za, D-Za and HC-D-Za groups; similarly, there was a decrease in IL-6 levels by -49, -38 and -41% in these same groups.

Alpha tumor necrosis factor (TNF-α , Homocysteine and C-reactive protein (CRP)

Based on the results, TNF-α plasma concentrations were reduced in HC-Za vs HC (-48%), D-Za vs D (-44%) and HC-D-Za vs HC-D (-48%) groups.

In addition, a reduction in homocysteine levels by -45, -32 and -52% was observed in HC-Za vs CH, D-Za vs D and HC-D-Za vs HC-D groups, respectively; similarly, CRP concentrations were reduced by -37, -25 and -38% in the same groups.

Rats were randomly divided into three groups (n=12). The first group was rendered hypercholesterolemic with an enriched cholesterol diet (1 %) (HC). The second group was rendered diabetic by intraperitoneal injection of streptozotocin (STZ) (35

Table 2 Erythrocytes TBARS (μmol/mL Hb), GSH (μmol/mL Hb) concentrations and antioxidant enzyme activities (U/g proteins).

Parameters	Groups HC	D	HC-D	HC- Za	D- Za	HC-D- Za
TBARS	142.5±3.2	166.1±3.5	109.2±6.4	80.06±1.7*	54.6±3.4≠	86.3±3.1$
GSH	8.3±1.5	11.4±2.2	6.4±1.5	13.4±2.2*	18.5±1.7≠	12.2±2.4$
SOD	180.2±4.2	225.3±3.9	105.5±3.6	275.2±2.3*	355.4±6.5≠	265.4±4.2$
GSH-Px	171.6±6.2	195.4±4.1	166.2±4.3	211.5±3.4*	271.6±5.1≠	208.5±3.5$
GSSH-Red	111.4±4.1	122.1±5.3	133.1±3.6	166.1±4.5*	88.1±3.4≠	155.2±5.01$
CAT	54.5±5.7	64.2±3.2	61.2±3.2	82.2±2.6*	105.2±4.1≠	85.2±4.1$

Data are shown as the mean ± SD for six values per group. In response to Z. album supplementation effects, comparisons between the six groups were performed by analysis of variance followed by Tukey honestly test. Differences with P<0.05 were considered statistically significant.
*HC-Za vs HC; Z. album effect, ≠D-Za vs D; Z. album effect, $HC-D-Za vs HC-D; Z. album effect

Table 3 Plasma inflammation marker concentrations.

Parameters	Groups HC	D	HC-D	HC-Za	D- Za	HC-D- Za
IL-1 (pg/mL)	16.2±0.1	18.3±0.4	17.3±0.1	8.7±0.4*	9.06±0.2≠	11.06±0.7$
IL-6 (pg/mL)	34.4±2.2	32.1±3.4	36.1±2.1	16.1±2.5*	18.7±3.3≠	20.2±2.1$
TNF- (pg/mL)	10.1±0.1	12.1±0.1	14.5±0.2	5.06±0.2*	6.6±0.2≠	7.3±0.2$
Homocystein (□mol/L)	13.3±0.1	12.3±0.1	18.1±0.1	7.1±0.1*	8.2±0.2≠	8.6±0.1$
CRP (mg/L)	32.2±1.2	30.5±1.8	43.2±1.3	20.1±0.9*	23.1±1.2≠	26.1±1.3$

Data are shown as the mean ± SD for six values per group. In response to Z. album supplementation effects, comparisons between the six groups were performed by analysis of variance followed by Tukey honestly test. Differences with P<0.05 were considered statistically significant.
*HC-Za vs HC; Z. album effect. ≠D-Za vs D; Z. album effect. $HC-D-Za vs HC-D; Z. album effect.

mg/kg of body weight) (D). The third group was hypercholesterolemic and diabetic (HC-D); each group was divided into two groups (n=6), untreated groups (HC, D, H C -D) and treated groups with 1 % Z. album extract (HC-Za, D-Za and HC-D-Za) for 4 weeks.

Note: Abbreviations: HC: Hypercholesterolemic rats group; HC-Za: Hypercholesterolemic rats group treated with *Zygophyllum album* (Z. album) aqueous extract (1%); D: Diabetic rats group; D-Za: Diabetic rats group treated with Z. album aqueous extract (1%); HC-D: Hypercholesterolemic-diabetic rats group; HC-D-Za: Hypercholesterolemic-diabetic rats group treated with Z. album aqueous extract (1%); IL-1 β: interleukin 1β ; IL-6 : interleukin 6 ; TNF-α Alpha tumoral necrose factor ; CRP : C-reactive protein.

Discussion

Oxidative stress is an underlying phenomenon of hypercholesterolemia [38]. Indeed, Lassoued et al., [39] reported that hypercholesterolemia is associated with the deterioration of antioxidant state, resulting from increased levels of lipid peroxidation markers and a decrease of some antioxidant enzymes activity. In addition, a strong link between oxidative stress and diabetes is undoubtedly accepted, resulting from radical hyperproduction associated with hyperglycemia and a decrease in antioxidant defenses [40]. Increased oxidative stress contributes to the development and progression of diabetes and its complications. It is suggested that persistent hyperglycemia leads to increased production of ROS, glucose self-oxidation and protein glycation, which lead to tissue damage [41].

Researches showed that free radical trappers and antioxidant available in plants are able to neutralize free radicals and therefore are useful in preventing complications of experimentally induced diabetes in a rat model [42].

In our study, the consumption of a cholesterol-enriched diet (1%) and the induction of experimental diabetes by STZ injection results in rat, an oxidative stress characterized by an increase in lipid peroxidation as well as a decrease in antioxidant enzymes activity.

Due to their O_2 high concentration subsequent to its transport, several studies have shown the sensitivity of RBC to oxidative stress [43]. In addition, the high exposure of RBC to ROS leads to elevated lipoperoxidation, morphological alterations and depletion of the enzyme antioxidant system [43]. Thus, lipid perox-

idation is directly related to the severity of hypercholesterolemia and permanent hyperglycemia.

In this study, the assessment of oxidative status in RBC after *Z. album* lyophilized aqueous extract supplementation presented a decrease in lipid peroxidation following the reduction of TBARS contents; conversely, GSH concentrations were increased. This lipid peroxidation reduction could be explained by an increased activity of some antioxidant enzymes; moreover, it could be suggested that Z. album lyophilized aqueous extract may have antioxidant properties that would probably be due to an inhibition of free radical production.

GSH, which is known for its powerful antioxidant power [44], protects not only against free radicals, but also against peroxides [45]. Stimulating the increase in GSH could be a response to the phytochemical content of the *Z. album* extract that would play an important role in antioxidant defense.

Authors have reported a reduction in GSH concentrations that appears to be directly related to GSH-Px activity, as GSH is a substrate and cofactor of GSH-Px. Diabetes-associated glycation can inactivate GSH-Px activity by blocking the active site of the enzyme [46]. Furthermore, treatment with Z. album extract decreases plasma glucose content [30], which leads to reduced glycation, resulting in a significant increase in GSH levels and GSH-Px activity. Moreover, an improvement in antioxidant enzymes activity of RBC was noted in this study, with an increase in SOD and CAT activities, as well as those of GSH-Px and GSSH-Red in all Z. album treated groups (HC-Za, D-Za and HC-D-Za). Zidan et al., [47] reported a decrease in TBARS levels and an increase in SOD and CAT activities, in RBC; however, GSH-Px and GSSH-Red activities remained similar in a cholesterol-enriched diet fed rats and treated with Portulaca oleracea lyophilized aqueous extract. Similarly, Berzou et al., [48] observed in RBC, that TBARS concentrations tended to decrease and SOD and CAT activities increased while GSH-Px and GSSH-Red activities remained similar, after treatment with the lyophilized aqueous extract of Zygophyllum gaetulum in a cholesterol-enriched diet fed rats (1%). The protective effect of Z. album extract could be explained by its ability to reduce the oxidative stress level with ROS production inhibition.

The increased SOD activity by Z. album extract suggest a significant elimination of $O^{2\bullet -}$ and, therefore, an inhibition of hydroxyl radical formation ($^{\bullet}OH^{-}$), which can protect RBC from their harmful effects. SOD can also convert O2• − to H_2O_2, which is decomposed into H_2O by GSH-Px and CAT. The phytochemical screening of Z. album revealed a flavonoids presence [28], known for their antioxidant effects and their roles in the capture of free radicals thus preventing lipid peroxidation. The beneficial effect of Z. album against oxidative stress could be correlated with the presence of these bioactive components. In treated, hypercholesterolemic, diabetic or hypercholesterolemic-diabetic, rats with Z. album lyophilized aqueous extract, the antioxidant defense system appears to be effective in RBC. This extract was therefore able to reduce lipids peroxidation and therefore prevent damage induced by oxidative stress.

Inflammation associated with hypercholesterolemia and diabetes is becoming progressively interesting because of its involvement in the atherosclerotic process and the development of CVD [49].

The secretion of cytokines such as TNF-α and-IL-6 by adipose tissue exacerbates insulin resistance and lipolysis that can increase glucose and VLDL production in the liver and thus strengthen insulin resistance in muscles. Furthermore, TNF-α production is increased by liver and adipocytic cells inflammation [50]. TNF-α derives mainly from adipose tissue and contributes to endothelial dysfunction and insulin resistance in both humans and rats [51, 52]. TNF-α mechanisms are not yet well elucidated but it has been suggested a decrease in the ARNm of GLUT4 and a reduction of the IRS receptor (Insulin Receptor Substrate) [53].

Our study clearly highlights the negative effect of hypercholesterolemia, diabetes and their association on inflammatory status in experimental rats since an increase in inflammation markers with increased circulating levels of CRP, TNF-α, IL-1β, IL-6 and homocysteine was noted. These abnormalities appear to be concomitant with increased cholesterol and blood glucose levels.

Our results were reliable with those obtained by Yadav et al., [54] who showed that the cholesterol-enriched diet induced, in Wistar rat, a systemic inflammation via a pro-inflammatory markers increase. Indeed, this had resulted in an increased synthesis of TNF-α and IL-6, which could be due to increased oxidative stress via activated immune cells [54].

It is well established that cholesterol causes inflammation, and oxidized cholesterol metabolites can be used to stimulate the inflammation markers expression [55]. In addition, homocysteine acts against cholesterol-induced inflammation in rats in vivo. This effect could be mediated by a process related to the tissue plasminogen activator [55]. Furthermore, [56] also showed that induction of experimental diabetes by deltamethrin in rats, leads to inflammation with high levels of CRP and TNF-α.

Our study showed reduced levels of CRP, TNF-α, IL-1β and IL-6 as well as homocysteine after *Z. album* treatment. These results converge with those obtained by Feriani et al., [56] who reported that Z. album ethanolic extract lead to a reduction of

inflammation that was shown by a decrease in CRP and TNF-α levels in diabetic rats. Moreover, Anvillea radiata lyophilized aqueous extract showed a strong anti-inflammatory activity in high-fat diet fed mice [57]. This suggests that Z. album extract polyphenolic compounds intake in diet seems to be useful in limiting systemic inflammation development. In fact, polyphenolic compounds have been identified in various plant species that have protective effects against inflammation [58]. Indeed, recent studies have reported that plant extracts may have dual use, hypoglycemic and anti-inflammatory [59], or could be used as hypolipemic treatments possibly acting on cellular pathways involved in the expression of adipogenesis [60].

Moreover, Kim et al., [61] have shown that flavonoids possess anti-inflammatory activity both in vitro and in vivo through the inhibition of IL-1β, IL-6 and TNF-α production. Crateva nurvala leaf ethanol extract also had a notable anti-inflammatory effect in rats with renal ischemia/reperfusion lesions because of its flavonoid content. Flavonoids express their anti-inflammatory activity, in part, by modulating the expression of pro-inflammatory genes such as cyclo-oxygenase-2, nitric oxides synthases and several cytokines.

Conclusion

In conclusion, it appears that the *Z. album* lyophilized aqueous extract has a beneficial effect in the prevention or treatment of erythrocytes oxidative stress and inflammation caused by hypercholesterolemia and diabetes, two major risk factors for cardiovascular disease.

Acknowledgments

This work was supported by the Algerian Ministry of Higher Education and Scientific Research.

Conflict of interest

The authors declare that there are no financial/commercial conflicts of interest.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

References

[1] Incalza MA, D'Oria R, Natalicchio A, Perrini S, Laviola L, Giorgino F. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. Vascular Pharmacology. 2018;100:1–19. Available from: https://dx.doi.org/10.1016/j.vph.2017.05.005.

[2] Hulsmans M, Holvoet P. The vicious circle between oxidative stress and inflammation in atherosclerosis. Journal of Cellular and Molecular Medicine. 2010;14(1-2):70–78. Available from: https://dx.doi.org/10.1111/j.1582-4934.2009.00978.x.

[3] Münzel T, Camici GG, Maack C, Bonetti NR, Fuster V, Kovacic JC. Impact of Oxidative Stress on the Heart and Vasculature. Journal of the American College of Cardiology. 2017;70(2):212–229. Available from: https://dx.doi.org/10.1016/j.jacc.2017.05.035.

[4] Keane KN, Cruzat VF, Carlessi R, de Bittencourt PIH, Newsholme P. Molecular Events Linking Oxidative Stress and Inflammation to Insulin Resistance andβ-Cell Dysfunction. Oxidative Medicine and Cellular Longevity. 2015;2015:1–15. Available from: https://dx.doi.org/10.1155/2015/181643.

[5] Bjørklund G, Chirumbolo S. Role of oxidative stress and antioxidants in daily nutrition and human health. Nutrition. 2017;33:311–321.

[6] Li H, Horke S, Förstermann U. Vascular oxidative stress, nitric oxide and atherosclerosis. Atherosclerosis. 2014;237(1):208–219. Available from: https://dx.doi.org/10.1016/j.atherosclerosis.2014.09.001.

[7] Bulua AC, Simon A, Maddipati R, Pelletier M, Park H, Kim KY, et al. Mitochondrial reactive oxygen species promote production of proinflammatory cytokines and are elevated in TNFR1-associated periodic syndrome (TRAPS). Journal of Experimental Medicine. 2011;208(3):519–533. Available from: https://dx.doi.org/10.1084/jem.20102049.

[8] Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011;469(7329):221–225. Available from: https://dx.doi.org/10.1038/nature09663.

[9] Patti ME, Corvera S. The Role of Mitochondria in the Pathogenesis of Type 2 Diabetes. Endocrine Reviews. 2010;31(3):364–395. Available from: https://dx.doi.org/10.1210/er.2009-0027.

[10] Wenzel P, Kossmann S, Münzel T, Daiber A. Redox regulation of cardiovascular inflammation – Immunomodulatory function of mitochondrial and Nox-derived reactive oxygen and nitrogen species. Free Radical Biology and Medicine. 2017;109:48–60. Available from: https://dx.doi.org/10.1016/j.freeradbiomed.2017.01.027.

[11] Chattopadhyay M, Khemka VK, Chatterjee G, Ganguly A, Mukhopadhyay S, Chakrabarti S. Enhanced ROS production and oxidative damage in subcutaneous white adipose

tissue mitochondria in obese and type 2 diabetes subjects. Molecular and Cellular Biochemistry. 2015;399(1-2):95–103. Available from: https://dx.doi.org/10.1007/s11010-014-2236-7.

[12] Kanikarla-Marie P, Jain SK. Role of Hyperketonemia in Inducing Oxidative Stress and Cellular Damage in Cultured Hepatocytes and Type 1 Diabetic Rat Liver. Cellular Physiology and Biochemistry. 2015;37(6):2160–2170. Available from: https://dx.doi.org/10.1159/000438573.

[13] He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. Cellular Physiology and Biochemistry. 2017;44(2):532–553. Available from: https://dx.doi.org/10.1159/000485089.

[14] Marik PE, Ronco C, Bellomo R, Kellum JA, Ricci Z. Chapter 76 - Endocrinology of the Stress Response During Critical Illness. Critical Care Nephrology. 2019;p. 446–54.

[15] Goldfine AB, Shoelson SE. Therapeutic approaches targeting inflammation for diabetes and associated cardiovascular risk. Journal of Clinical Investigation. 2017;127(1):83–93. Available from: https://dx.doi.org/10.1172/jci88884.

[16] Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. Journal of Clinical Investigation. 2017;127(1):1–4. Available from: https://dx.doi.org/10.1172/jci92035.

[17] Liese AD, Ma X, Ma X, Mittleman MA, The NS, Standiford DA, et al. Dietary quality and markers of inflammation: No association in youth with type 1 diabetes. Journal of Diabetes and its Complications. 2018;32(2):179–184. Available from: https://dx.doi.org/10.1016/j.jdiacomp.2017.10.015.

[18] Colina-Coca C, González-Peña D, de Ancos B, Sánchez-Moreno C. Dietary onion ameliorates antioxidant defence, inflammatory response, and cardiovascular risk biomarkers in hypercholesterolemic Wistar rats. Journal of Functional Foods. 2017;36(7):300–309. Available from: https://dx.doi.org/10.1016/j.jff.2017.07.014.

[19] Zanchet B, Gomes DB, Corralo VS, Diel KAP, Schönell AP, Faust C, et al. Effects of hydroalcoholic extract of Celtis iguanaea on markers of cardiovascular diseases and glucose metabolism in cholesterol-fed rats. Revista Brasileira de Farmacognosia. 2018;28(1):80–91. Available from: https://dx.doi.org/10.1016/j.bjp.2017.12.001.

[20] Chávez-Sánchez L, Espinosa-Luna JE, Chávez-Rueda K, Legorreta-Haquet MV, Montoya-Díaz E, Blanco-Favela F. Innate Immune System Cells in Atherosclerosis. Archives of Medical Research. 2014;45(1):1–14. Available from: https://dx.doi.org/10.1016/j.arcmed.2013.11.007.

[21] A report of the consultation meeting on traditional and modern medicine: Harmonizing two approaches. Beijing, Geneva; 2015. Available from: http://iris.wpro.who.int/handle/10665.1/5573.

[22] Bouasla A, Bouasla I. Ethnobotanical survey of medicinal plants in northeastern of Algeria. Phytomedicine. 2017;36:68–81. Available from: https://dx.doi.org/10.1016/j.phymed.2017.09.007.

[23] Taika BB, Bouckandou M, Souza A, Bourobou HPB, MacKenzie LS, Lione L. An overview of anti-diabetic plants used in Gabon: Pharmacology and toxicology. Journal of Ethnopharmacology. 2018;216:203–228. Available from: https://dx.doi.org/10.1016/j.jep.2017.12.036.

[24] Azzi R, Djaziri R, Lahfa F, Sekkal FZ, Benmehdi H, Belkacem N. Ethnopharmacological survey of medicinal plants used in the traditional treatment of diabetes mellitus in the North Western and South Western Algeria. The Journal of Medicinal Plants Research. 2012;6(10):2041–2050.

[25] Bedekar A, Shah K, Koffas M. Natural products for type II diabetes treatment". Advances in applied microbiology. 2010;71.

[26] Achi NK, Ohaeri OC, Ijeh II, Eleazu C. Modulation of the lipid profile and insulin levels of streptozotocin induced diabetic rats by ethanol extract of Cnidoscolus aconitifolius leaves and some fractions: Effect on the oral glucose tolerance of normoglycemic rats. Biomedicine & Pharmacotherapy. 2017;86:562–569. Available from: https://dx.doi.org/10.1016/j.biopha.2016.11.133.

[27] El-Sayyad HIH, Elmansi AA, Bakr EHM. Hypercholesterolemia-induced ocular disorder: Ameliorating role of phytotherapy. Nutrition. 2015;31(11-12):1307–1316. Available from: https://dx.doi.org/10.1016/j.nut.2015.05.013.

[28] Belmimoun A, Meddah B, Elarbi S, Sonnet K, P. Phytochemical study of Zygophyllum album extract. International Journal of Engineering Management. 2017;4(5).

[29] Ghoul JE. Vasorelaxant Effects of Aqueous Extract of Zygophyllum Album and Antihyperglycemic Activities in Streptozotocin-Induced Diabetic Mice. Journal of Diabetes & Metabolism. 2014;05(09). Available from: https://dx.doi.org/10.4172/2155-6156.1000426.

[30] Bahlil Y, Krouf D, Taleb-Dida N. Zygophyllum album aqueous extract reduces glycemia and ameliorates lipid profile and oxidative damage in hypercholesterolemic-diabetic rats. Nutrition & Food Science. 2019;49(1):141–157. Available from: https://dx.doi.org/10.1108/nfs-03-2018-0078.

[31] Council instructions about the protection of living animals used in scientific investigations. Official J1987 L358 of 18-

12-1986. Corrigendum Official J;117:5–05.

[32] Wang YM, Zhang B, Xue Y, Li ZJ, Wang JF, Xue CH, et al. The mechanism of dietary cholesterol effects on lipids metabolism in rats. Lipids in Health and Diseases. 2010;9:4–9.

[33] Islam MS, Wilson RD. Experimentally induced rodent models of type 2 diabetes. Methods in Molecular Biology. 2012;933:161–174.

[34] Zhang M, Lv X, Li J, Meng Z, Wang Q, Chang WG, et al. Sodium caprate augments the hypoglycemic effect of berberine via AMPK in inhibiting hepatic gluconeogenesis. Molecular and cellular endocrinology. 2012;363(1-2).

[35] Quintanilha AT, Packer L, Davies JMS, Racanelli TL, Davies KJA. MEMBRANE EFFECTS OF VITAMIN E DEFICIENCY: BIOENERGETIC AND SURFACE CHARGE DENSITY STUDIES OF SKELETAL MUSCLE AND LIVER MITOCHONDRIA. Annals of the New York Academy of Sciences. 1982;393(1 Vitamin E):32–47. Available from: https://dx.doi.org/10.1111/j.1749-6632.1982.tb31230.x.

[36] Brown JM, Grosso MA, Terada LS, Beehler CJ, Toth KM, Whitman GJ, et al. Erythrocytes decrease myocardial hydrogen peroxide levels and reperfusion injury. American Journal of Physiology-Heart and Circulatory Physiology. 1989;256(2):H584–H588. Available from: https://dx.doi.org/10.1152/ajpheart.1989.256.2.h584.

[37] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical Biochemistry. 1968;25(1):192–205. Available from: https://dx.doi.org/10.1016/0003-2697(68)90092-4.

[38] Moreira ARS, Olivero-David R, Vázquez-Velasco M, González-Torres L, Benedí J, Bastida S, et al. Protective Effects of Sea Spaghetti-Enriched Restructured Pork Against Dietary Cholesterol: Effects on Arylesterase and Lipoprotein Profile and Composition of Growing Rats. Journal of Medicinal Food. 2014;17(8):921–928. Available from: https://dx.doi.org/10.1089/jmf.2013.0100.

[39] Lassoued I, Trigui M, Ghlissi Z, Nasri R, Jamoussi K, Kessis M, et al. Evaluation of hypocholesterolemic effect and antioxidant activity of Boops boops proteins in cholesterol-fed rats. Food Funct. 2014;5(6):1224–1231. Available from: https://dx.doi.org/10.1039/c3fo60705d.

[40] Zein S, Rachidi S, Hininger-Favier I. Fer, stress oxydant et diabète gestationnel. Médecine des Maladies Métaboliques. 2013;7(2):144–148. Available from: https://dx.doi.org/10.1016/s1957-2557(13)70511-x.

[41] Ezuruike UF, Prieto JM. Review: The Use of Plants in the Traditional Management of Diabetes in Nigeria: Pharmacological and Toxicological Considerations. Journal of Ethnopharmacology. 2014;155:857–924.

[42] Okamoto T, Gohil K, Finkelstein EI, Bove P, Akaike T, van der Vliet A. Multiple contributing roles for NOS2 in LPS-induced acute airway inflammation in mice. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2004;286(1):L198–L209. Available from: https://dx.doi.org/10.1152/ajplung.00136.2003.

[43] Giustarini D, Colombo G, Garavaglia ML, Astori E, Portinaro NM, Reggiani F, et al. Assessment of glutathione/glutathione disulphide ratio and S-glutathionylated proteins in human blood, solid tissues, and cultured cells. Free Radical Biology and Medicine. 2017;112:360–375. Available from: https://dx.doi.org/10.1016/j.freeradbiomed.2017.08.008.

[44] Giustarini D, Galvagni F, Donne ID, Milzani A, Severi FM, Santucci A, et al. N-acetylcysteine ethyl ester as GSH enhancer in human primary endothelial cells: A comparative study with other drugs. Free Radical Biology and Medicine. 2018;126:202–209. Available from: https://dx.doi.org/10.1016/j.freeradbiomed.2018.08.013.

[45] Fournet M, Bonté F, Desmoulière A. Glycation Damage: A Possible Hub for Major Pathophysiological Disorders and Aging. Aging and disease. 2018;9(5):880–880. Available from: https://dx.doi.org/10.14336/ad.2017.1121.

[46] Zidan Y, Bouderbala S, Bouchenak M. Portulaca oleracea aqueous extract reduces oxidative stress in erythrocytes and tissues, in rats fed enriched-cholesterol diet. Journal of Experimental and Integrative Medicine. 2016;6(1):21–21. Available from: https://dx.doi.org/10.5455/jeim.260116.or.145.

[47] Berzou S, Krouf D, Dida N, Taleb-Senouci D, Guenzet A. Effects of Zygophyllum gaetulum Aqueous Extract on Erythrocytes Lipid Peroxidation and Paraoxonase 1 Activity in Hypercholesterolemic Rats. International Journal of Pharmacognosy and Phytochemical Research. 2016;8(10):1689–1694.

[48] Magnuson A, Fouts J, Booth A, Foster M. Obesity-induced chronic low grade inflammation: Gastrointestinal and adipose tissue crosstalk. Integrative Obesity and Diabetes. 2015;1(5):103–108. Available from: https://dx.doi.org/10.15761/iod.1000124.

[49] Choi JJ, Park MY, Lee, Hj, Yoon DY, Lim Y, et al. TNF-α increases lipogenesis via JNK and PI3K/Akt pathways in SZ95 human sebocytes. The Journal of Dermatological Science. 2012;65(3):179–188.

[50] Mm RA, Sánchez-Roitz C. Tumor necrosis factor-α, insulin resistance, the lipoprotein metabolism and obesity in humans. Nutrición Hospitalaria. 2012;27(6):1751–1757.

[51] Yida Z, Imam MU, Ismail M, Ismail N, Ideris A, Abdullah MA. High fat diet-induced inflammation and oxidative stress are attenuated by N-acetylneuraminic acid in rats. Journal of Biomedical Science. 2015;22(1). Available from: https://dx.doi.org/10.1186/s12929-015-0211-6.

[52] Nieto-Vazquez I, Fernández-Veledo S, Krämer DK, Vila-Bedmar R, Garcia-Guerra L, Lorenzo M. Insulin resistance associated to obesity: the link TNF-alpha. Archives of Physiology and Biochemistry. 2008;114(3):183–194. Available from: https://dx.doi.org/10.1080/13813450802181047.

[53] Yadav R, Khan SH, Mada SB, Meena S, Kapila R, Kapila S. Consumption of Probiotic Lactobacillus fermentum MTCC: 5898-Fermented Milk Attenuates Dyslipidemia, Oxidative Stress, and Inflammation in Male Rats Fed on Cholesterol-Enriched Diet. Probiotics and Antimicrobial Proteins. 2019;11(2):509–518. Available from: https://dx.doi.org/10.1007/s12602-018-9429-4.

[54] Pirchl M, Ullrich C, Sperner-Unterweger B, Humpel C. Homocysteine has anti-inflammatory properties in a hypercholesterolemic rat model in vivo. Molecular and Cellular Neuroscience. 2012;49(4):456–463. Available from: https://dx.doi.org/10.1016/j.mcn.2012.03.001.

[55] Feriani A, Hachani R, Kaabi B, Ncir M, Feki AE, Allagui MS. Protective effects of Zygophyllum album extract against deltamethrin-induced hyperglycemia and hepatopancreatic disorders in rats. Canadian Journal of Physiology and Pharmacology. 2016;94(11):1202–1210. Available from: https://dx.doi.org/10.1139/cjpp-2016-0132.

[56] Kandouli C, Cassien M, Mercier A, Delehedde C, Ricquebourg E, Stocker P, et al. antioxidant and anti-inflammatory properties of water and n-butanol soluble extracts from Saharian Anvillea radiata in high-fat-diet fed mice. Journal of Ethnopharmacology. 2017;207:251–267.

[57] Sreeja PS, Arunachalam K, de Oliveira Martins DT, da Silva Lima JC, Balogun SO, Pavan E, et al. Sphenodesme involucrata var. paniculata (C.B. Clarke) Munir.: Chemical characterization, anti-nociceptive and anti-inflammatory activities of methanol extract of leaves. Journal of Ethnopharmacology. 2018;225:71–80. Available from: https://dx.doi.org/10.1016/j.jep.2018.06.035.

[58] Heyman L, Axling U, Blanco N, Sterner O, Holm C, Berger K. Evaluation of Beneficial Metabolic Effects of Berries in High-Fat Fed C57BL/6J Mice. Journal of Nutrition and Metabolism. 2014;2014(3):1–12. Available from: https://dx.doi.org/10.1155/2014/403041.

[59] Lee IS, Kim DY, Choi BY. Antioxidative activity of blueberry leaf extract prevents high-fat induced obesity in C57BL/6 mice. European Journal of Cancer Prevention. 2014;19(3):209–215.

[60] Kim HP, Son KH, Hw C, Kang SS. Anti-inflammatory Plant Flavonoids and Cellular Action Mechanisms. Journal of Pharmacological Sciences. 2004;96(3):229–245.

[61] Choucry MA, Khalil MNA, Awdan SAE. Protective action of Crateva nurvala Buch. Ham extracts against renal ischaemia reperfusion injury in rats via antioxidant and anti-inflammatory activities. Journal of Ethnopharmacology. 2018;214:47–57. Available from: https://dx.doi.org/10.1016/j.jep.2017.11.034.