IJBPAS, December, 2017, 6(12): 2414-2430

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTIHYPERLIPIDEMIC ACTIVITY OF *ZYGOPHYLLUM SIMPLEX* ETHANOLIC EXTRACT ON HIGH CHOLESTEROL DIET-INDUCED HYPERLIPIDEMIA IN RATS

HAYTHAM M. DARADKA[1*], KAMAL M.S.MANSI[2], BANDAR R. ALSEHLI[3], ABDUL MANNAN MOHAMED[4], YASER S. BINNASER[1], [5]MUWAFFAG BADAWNEH [5] AND JALAL ALJAMAL [5]

[1]Haytham M. Daradka, Biology Department, Faculty of Science, Taibah University, Al-Madinah Al-Munawwarah, 41321, Saudi Arabia

[2]Department of Physiology, Taibah University, Al-Madinah Al-Munawwarah, Saudi Arabia

[3]Bandar R. Alsehli, Chemistry Department, Faculty of Science, Taibah University, Al-Madinah Al-Munawwarah, 41321, Saudi Arabia

[4]Abdul mannan Mohamed, Medical Center, Taibah University, Al-Madinah Al-Munawwarah, 41321, Saudi Arabia

[1]Yaser S. Binnaser, Biology Department, Faculty of Science, Taibah University, Al-Madinah Al-Munawwarah, 41321, Saudi Arabia.

[5]Muwaffag Badawneh, Faculty of Pharmacy, Jerash University, Jerash-Jordan

[5]Jalal Aljamal, Faculty of Pharmacy, Jerash University, Jerash-Jordan

***Corresponding Author: Dr. Haytham M. Daradka, Associate Professor Department of Biological Sciences, Faculty of Science, Taibah University, Al-Madinah Al-Munawwarah, 41321, Saudi Arabia, Phone: +966 535923936. E-mail: hmdaradka@yahoo.com**

Received 25th Feb. 2017; Revised 10th April 2017; Accepted 23rd July 2017; Available online 1st Dec. 2017

ABSTRACT

Objective: Aerial parts of *Zygophyllum simplex* are often used for the medical treatment of skin's horny patches and as an analgesic, anti-inflammatory and anthelmintic due to its unique bioactivities.

Method: The antihyperlipidemic and antioxidant effects of the ethanolic extract of *Z. simplex* leaves in high-fat diet (HFD) induced hyperlipidemic rats were examined. Fifty male Wistar rats (divided in five diet groups of 10) were adapted to a normal standard diet (NSD) or a high fat diet (HFD) with or without the treatment of *Z. simplex* samples for 30 days.

Results: The treatments at doses of (250 and 500 mg/kg) oral administration of *Z. simplex* to hyperlipidemia rats were appreciably effective in decreasing the levels of serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), triacylglycerols (TG) and tissue lipid accumulation while increasing the levels of serum high-density lipoprotein-cholesterol (HDL-c), adjusting the metabolic disturbance of lipoprotein and increasing the antioxidant enzyme activity and repressing the development of atherosclerosis.

Conclusion: our findings suggest that *Z. simplex* could be of great therapeutic potential for the treatment of hyperlipidemia disease and offers a promising new natural additive to food or water to relieve illness or prevent disease.

Keywords: *Zygophyllum simplex*, anti-hyperlipidemic activity, triglyceride, cholesterol, high cholesterol diet induced hyperlipidemia

1. INTRODUCTION

The disease of coronary artery is among the primary reason for mortality and morbidity worldwide. A report released in 2012 revealed that over three million deaths or 26% of total deaths per year have been associated with this condition [1,2]. The major risk factors for the development of cardiovascular disease are hyper triglyceridemia and hypercholesterolemia. Thus, it has been found that reducing the cholesterol level in plasma has an effective role in treating atherosclerosis [3,4].

Numerous chemical drugs, for instance statins and fibrates, are categorized by a good efficacy and a high lipid -lowering speed [5]. Nevertheless, the demand for treatment using these chemicals cannot be met because of the diversity of hyperlipidemia patients, some potential adverse effects and the possibility of patients to become drug dependence [6]. In contrast, plant materials and its extracts have been distinguished by their negligible side effects and several targets in preventing and curing hyperlipidemia [7].

Plants with their different varieties and active constituents have been applied extensively in several countries as folklore medicines and traditional remedies for the reputable treatment of hyperlipidemias. Recently, researchers are continuously focusing on finding appropriate antihyperlipidemic agents from plants as they are less toxic than the presently used drugs, easily absorbed and readily available [4,8].

The Zygophyllum genus is the major genus of Zygophyllaceae which comprises around 100 known species from the area around Mediterranean Sea to Middle Asia, South Africa and Australia regions [9]. The aerial parts of *Zygophyllum simplex* are often used for the medical treatment of skin's horny patches and as an analgesic, anti-inflammatory and anthelmintic due to its unique bioactivities. It comprises under-shrubs or perennial shrubs with succulent cylindrical seldom flattened leaves, 1-2 foliate or simple [10,11].

The Seven types of Zygophyllum that grow in Saudi Arabia are Zygophyllum album, Z. migahidii, Z. gaetulum, Z. coccineum, Z. decumbens, Z. mandavillei and Z. simplex [12]. Zygophyllum simplex Linn completes its entire life cycle within the space of a year and it is 8-20 cm tall, suberect to procumbent, glabrous herb and profusely branched. Its branches and stem are purplish or pale-green, minutely striate, leaves simple and succulent; seeds fusiform and smooth. It is spread in Saudi Arabia, Pakistan, Rajasthan, Egypt, Kutch area of Gujarat and South-West Africa [13,14]. The juice extracted from Z. simplex serves as an antiseptic and in the treatment of skin horny patches [15,16]. Zygophyllum simplex may be used as an analgesic and its seeds are anthelmintic [17]. anti-inflammatory [18], and hepatoprotective [19].

Since that, the goal of this study was to investigate the antihyperlipidemic and antioxidant effects of the ethanolic extract of *Z. simplex* leaves in high-fat diet (HFD) induced hyperlipidemic rats.

2. MATERIALS AND METHODS

2.1 Plant material

During the flowering stage in Al-Madinah Al-Munawwarah, fresh aerial parts of Z. simplex L. (Zygophyllaceae) were collected. Prof. Soliman Haroun from Floriculture and Medicinal plants, Department of Biology, Faculty of Science, Taibah University, Al-Madinah Al-Munawwarah, Saudi Arabia established the identity of the plants. The ethanolic extract of Z. simplex was prepared by soaking 100 g air dried powder of aerial plant parts in 1 L of 70% ethanol at 45 ºC for 2 days. Next, to remove particulate matters the mixture was filtered and lyophilized. This resulted

in a powder of (22 g) which was stored at -20 °C until usage [20,21].

The chemical components of aqueous extract of Z. simplex were separated and screened using a thin layer chromatography according to the specification method of Wagner and Bladt method [22,23]. A standard reference solution containing quercetin (Merck, USA), eucalyptol (Fluka), rutin (Fluka, USA), menthol (Fluka, USA), chlorogenic acid (Merck, USA), hiperoside (Merck, USA), coumarin (Fluka, USA) and caffeic acid (Merck, USA) was prepared.

2.2 Experimental animal

Before the experiments were initiated on animals an ethical approval was acquired from the Institutional Animal Care and Use Committee (IACUC) outlined by Faculty of Science, Taibah University, Saudi arabia (6/1437). Fifty mature of male Wistar rats (Rattus norvegicus) that weigh 200-250 g were offered by the animal house. The rats were separately housed in cages and preserved under restrained environmentally friendly conditions with relative humidity (45-55%), temperature (20±2 EC) and 12 hours light/dark cycle. All rats were fed, under strict hygienic conditions and set criteria, with water ad libitum and rodent pellet diet after a week of acclimatization [24,25]. The rats were arbitrarily distributed into three various groups, for each having ten rats.

2.3 Determination of LD_{50}

The calculation of LD_{50} represents the potential acute toxicity of plants dose which can be lethal to 50% of any rats group [26]. In this experiment, the LD_{50} in rats was experimented to evaluate the suitable dose that should be applied. Thus, 24 albino rats were dispersed into 4 different groups each one contained 6 rats (300 g). Each dosage of ethanolic extract of Z. simplex aerial part viz., 200, 400, 600, 1000, 3000, 4000 and 5000 µg was dissolved in a 0.2 mL of a normal brine solution of 0.9% NaCl and directed intraperitoneally to every corresponding rat in a group. Then, in transparent plastic cages rats were housed and monitored for any toxic symptoms under temperature control (24°C) for 24 h. The quantity of dead rats was counted in every group after 24 h and the mortality percentage was determined. Six controlled rats which received only 1 mL of distilled water through an intraperitoneal infusion were compared with treated rats under the same experimental condition [24,27,28].

2.4 Experimental design

Before the experiments were established, rats under the experimental condition were fed with a basic diet for a week. After adaptation, ten rats were randomly

nominated as the normal control group (NC). These were fed with a basic diet as a standard chow diet where the others fed with a high-fat diet HFD as presented in Table 1.

This consisting of standard chow supplemented with cholesterol (350 mg/kg/day) (Oxford Lab, Mumbai, India), dissolved in cottonseed oil for 30 days. This dose was proved to induce hypercholesterolemia [29]. On the other hand, cotton seed oil is natural oil that contains a high proportion of polyunsaturated fatty acids and proved to have no effects on the tissues [30].

Hypercholesterolemic rats were, then, divided by random into four treatment groups according to the following schedule:

Group 1 (control group): fed a standard pellet 100 g/kg/d.

Group 2 (HCD): fed a HCD and received no additional treatment.

Group 3 (SIM): HCD received simvastatin at doses of 10 mg/kg (23Zhang et al., 2009).

Group 4 (EA 250): HCD received 250 mg/kg *Z. simplex* extract

Group 5 (EA 500): HCD received 500 mg/kg *Z. simplex* extract

During treatment, all animals continued to receive the HCD. Animals were treated once daily for 30 days by oral gavages. After 12 h of fasting, from the completion time of the experiment, chloral hydrate was used to anesthetize animals via intra-abdominal approach. To avoid circadian in total groups, animals were sacrificed at the same period of the day [31].

Cardiac puncture was used to collect blood samples when after 30 min from collection they were centrifuged at 2000×g for 15 min and stored at -20 EC until usage. The liver, heart and aorta were gathered and then washed from fats. Then, they were adhered to the connective tissue, weighed and kept at a proper storage condition at -20 ºC until usage. Blood samples were centrifuged at 3000 rpm for 15 min to obtain serum [32].

2.5 Body and organ weights

Measurement of body weight changes (Δwt) of rats in each group were calculated and expressed in percentage (%) as shown:

(Body weight on day 30-body weight on day1)/(Body weight on day 1) 100. The absolute liver weight of each rat group was measured using an electronic weighing balance, from which the relative liver weight per 100 g body weight of rat was calculated according to the following equation : [Weight of rat liver (g)/body weight on day 30 (g)] 100.

2.6. Blood biochemical analysis

Serum obtained was used to examine the following:

The levels of serum triglycerides (TG), total cholesterol (TC), high-density

lipoprotein-cholesterol (HDL-c) and low-density lipoprotein-cholesterol (LDL-c) were determined according to the manufacturer's instructions using technical kits (purchased from Jian cheng Biotech. Sci. Inc., Nanjing, China). The following equation was used to calculate the arteriosclerosis index (AI): AI = TC-HDL-c /HDL-c.

2.7. Statistical analysis:

Statistical Package of Social Sciences (SPSS), version 17 program was used to analyse data which expressed as mean ± SEM (Chicago, IL, USA). Comparisons between groups for all parameters were conducted using Dunnat's multiple comparison tests followed by one-way analysis of variance (ANOVA) [33]. The reported P values were two-tailed ($P < 0.05$) considered significant.

3. RESULTS

3.1. Phytochemical study

Seventeen analytes belonging to different chemical classes were isolated from the aerial parts of Z. simplex using the phytochemical methods. Preliminary screening of the isolated compounds exposed the existence of flavonoids, saponins and alkaloids as main constituents. The existence of triterpenes and/or sterols, coumarins, glycosides and/or carbohydrates, cardiac glycosides and/or tannins was also recorded.

3.2. Toxicity study

The extract of Zygophyllum simplex showed a non-toxic nature of lower doses until reaching the level of 1000 mg/kg. Throughout the entire period of the experiment, rats did not display any drug-induced physical symptoms of toxicity and no deceases were observed [34].

3.3. Effect of Z. simplex ethanolic extract on body weight

The body weight changes and relative liver weight of the studied rats after 30 days were shown in Table 2. HD caused increasing in the body weight of the studied rats which could be seen from the relatively higher weight gained among all the rats fed with HD either with or without treatment (Group 2- Group 5). However, no significant difference ($P > 0.05$) in weight gained among all the HD fed rat groups with the normal standard diet control rats was observed except for Group 3. Treatment of 40 mg/ kg/ day of simvastatin caused a significant weight gained in the studied rats; which was significantly higher ($P > 0.05$) than the normal standard diet control rats.

The reduction in the body weights was not significant in rats nourished with cholesterol diet and later treated with SIM and Z. simplex extract (Groups 4 and 5) compared to the primary body weights.

liver weight had increased considerably in cholesterol fed rats (Table 2).

3.4. Effect of Z. simplex extracts on serum lipid profile and atherosclerosis index

In the present study, the results obtained from exclusive high cholesterol fed rats were compared with those of the normal group. The values of the groups that were fed HCD plus extract of *Z. simplex* leaves were compared with those of HCD group. In Table 3, a significant increased was observed in serum cholesterol in treated rats fed with atherogenic diet. It also shows a significant increase in TC, TG and LDL-C while a non-significant decrease was observed in HDL-C in HCD group as compared to control group.

SIM ameliorated significantly dyslipidemia whereas serum HDL-C slightly increased in rats treated with SIM. On the other hand, Zygophyllum simplex reduced the serum level of LDL-C, TG and TC in comparison to HCD group. Additionally, the difference in the calculated atherosclerosis index between the treated groups and the HCD group was significant ($P< 0.05$).

3.5. Effect of Z. simplex extracts on liver

Nourishing of the HCD for 8 weeks brought about a non-noteworthy increment in the levels of serum of ALP, AST and ALT when compared with the normal control group. SIM and *Zygophyllum simplex* extracts showed non-significant changes in these levels (Table 4). Hypercholesterolemic rats also showed a non-significant change in serum creatinine, BUN and total protein when compared to normal controls. In addition, non-significant changes in serum levels were observed in SIM and *Zygophyllum simplex* extracts which were orally administered (Table 4).

4. DISCUSSION

Male Wistar rats were used in the *in vivo* cholesterol-lowering assay was done to avoid the effects of hormonal factors in developing metabolic diseases, i.e., type 2 diabetes and insulin resistance [35]. The 10 mg/kg bw was the chosen dose of the Simvastatin as adopted from the established protocol for the determination of lipid-lowering property of plant samples in rats [36]. The 250 mg/kg bw served as the lower dose limit, while the 500 mg/kg bw was the upper limit dose. The serum cholesterol, triglycerides, low-density lipoproteins and high-density lipoproteins were monitored weekly for two weeks to determine if the effect of the crude extract is time-dependent [37,38].

Cholesterol feeding has often been used to increase serum lipid levels mainly TC, TG and LDLc along with decrease in HDLc levels are known to cause hyperlipidemia with progression to arteriosclerosis to study

the etiology of hyperlipidemia-related metabolic disturbances [39].

Feeding of HD to the studied animals caused significant rise of serum TC and LDL-c compared to control rats. This was related with the extreme burden of cholesterol to the liver, which exceeded the normal physiological limits, causing the inability of liver to metabolize the lipids. Thus, resulting in the returned of high cholesterol in the circulating blood [40].

Consequently, the accumulation of fatty acid in the hepatocyte caused an increase in the relative liver weight [41]. This can be seen from the significant higher relative liver weight on the HD induced groups (both untreated and treated) in comparison to the control group. Similarly, the body weights gained in the HD groups were shown to be higher than the control diet group. HD fed to the studied rats induced greater body fat deposition because of the increase of adipocyte number and size [42]. The HD groups, which treated with *Z. simplex* ethanolic extract, were shown to have relatively lower relative liver weight and lower body weight gained. These indicated that the extracts of *Z. simplex* ethanolic were capable to prevent the accumulation of fats in the body and liver [43].

The observation was in accordance with Kumar et al., (2010) which stated that HCD had a significant increase in the body weight [42]. HCD treatment induced hyperlipidemia evidenced by increase in the mean relative weights of liver might be due to excessive accumulation of lipids [44,45].

Dietary administration of cholesterol increases considerably the hepatic concentrations of the TG and TC in rats indicating hepatic lipid metabolism disturbance. The level of Hepatic TG is predominantly controlled by beta-oxidation, secretion and TG synthesis in the lipoprotein's form [46]. The accumulation of Hepatic TG by high dietary cholesterol is participated in the TG synthesis and fatty acid stimulation in rats. It is also assigned to the reduction of beta-oxidation of fatty acid, afflux and secretion of LDL [47]. Treatment with Z. simplex extract, at two different doses (250, 500 mg/kg) considerably decreased the levels of increased serum lipid parameters in relation to the control group. The Z. simplex extract showed protective action at 500 mg/kg dose and demonstrated a substantial decrease in the raised HCD induced serum levels of TC, LDL-c and triglycerides. It is broadly recognized that the serum reduction in HDL is a danger element for atherosclerosis development. HDL enables the cholesterol translocation from the peripheral tissue, such as arterial walls to

liver for catabolism. The rise in HDL might decelerate the process of atherosclerotic. Increased levels of HDL (cardio protective lipid) after administration of Z. simplex extracts concluded that the extract is a powerful cardio protective agent and this effect may be as a result of the increase in the activity of lecithin-cholesterol acyl transferase (LCAT), that play a main part in combining the free cholesterol into HDL which is repossessed by the liver cells. Numerous studies indicated that an increase in HDLc is related with a reduction in coronary risk as high levels of LDLc and TC are key coronary risk factors. Treatment of Z. simplex extract lowered both TC and LDLc in HCD induced hyperlipidemic rats. The higher the AI, the more is the risk of the cardiovascular disease [48]. AI shows the deposition of plaque, foam cells or the infiltration of fatty lipids in coronaries, heart, aorta, kidneys and liver. The increase in AI in hyperlipidemic rats enhances the endothelial dysfunction and the cardiovascular pathogenesis probability. AI in HCD treated animals were increased moderately. Significant decrease in AI value was observed in *Z. simplex* extract supplemented animals which might suggest the atheroprotective/cardio protective potential of *Z. simplex* extract [49].

These results indicated the cholesterol lowering activity of the *Z. simplex* flavonoid against HCD which can be as a result of rapid catabolism of LDLc via its hepatic receptors for last elimination in the form of bile acids as revealed [50]. Flavonoids have displayed a diversity of pharmacological activities, containing the antioxidant effect and antiatherogenesis [51].

In this study, serum aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities were substantially high in high-cholesterol fed diet than in normal rats. These results were in agreement with those of Sudhahar et al. (2007) [52]. The intake of High fat diet produced a very significant elevated creatinine and serum urea concentrations of hypercholesterolemic control rats in comparison to normal rats (Table 4). Accelerated amino acid deamination and enhanced protein catabolism for gluconeogenesis is probable, a tolerable postulate to interpret the urea elevated levels, where elevated creatinine concentration level is linked with abnormal renal function [53]. Along these lines, the cholesterol-enriched diet improved excretion of indicative parameters of renal dysfunction for instance creatinine and urea. These results agreed with earlier studies which indicated that

hypercholesterolemia encourages glomerular injury [54,55].

It is always beneficial to use a drug that decreases serum total cholesterol, low-density lipoprotein and triglycerides levels and elevates serum HDL levels [56]. Finding a drug that can produce such changes in cholesterol is of prime importance. However, this study showed that cholesterol levels only serve as a surrogate indicator for the development of atherosclerosis and other related cardiovascular conditions. Therefore, a comprehensive investigation about the direct effect of the crude extract on the formation of atherosclerosis must be given focus in future studies [57].

Table 1: Compositions (%) of the experimental diets

Ingredient	Basic diet	High-fat diet
Corn meal	30	26.3
Soybean meal	20	17.5
Wheat bran	25	21.9
Wheat flour	16	14
Fish meal	5	4.4
Bone meal	2	1.8
Yeast powder	1	0.9
NaCl	1	0.9
Cholesterol	0	2
Lard	0	10
Sodium cholate	0	0.3

Table 2 Effect of different treatments on the percentage body weight changes (% ∆wt) and relative liver weight in the studied rats

Group	% ∆wt	Relative liver weight
Control	11.26 ± 2.04 b	3.36 ± 0.25 bc
HCD	19.93 ± 3.89 ab	4.91 ± 0.71a
HCD + SIM 10 mg/kg	23.89 ± 3.61 a	4.17 ± 0.37abc
HCD + *Z. simplex* 250 mg/kg	13.31 ± 2.22 b	3.93 ± 0.61a
HCD + *Z. simplex* 500 mg/kg	16.44 ± 2.71 b	3.97 ± 0.27 abc

Data are mean ± SEM (n=10) with different superscript letters abc within the same column indicate significant differences (P<0.05)

Table 3: Effects of *Z. simplex* on serum lipid levels of experimental hyperlipidemia rats induced by HFD for 30 days

Group	TC	TG	Phospholipid	HDL-c	LDL-c	V LDL-c	AI
	mg/dL	mg/dL	mg/dL				
Control	116.38 ± 7.11	69.05 ±6.76	161.41 ± 4.14	42.34 ± 3.45	102.35 ± 4.91	13.81 ±1.25	1.74 ±0.25
HCD	241.91 ± 8.35	171.95 ±3.46	247.22 ± 4.33	32.27 ± 1.81	207.35 ± 3.17	34.39 ±2.60	6.49 ±0.60
HCD + SIM 10 mg/kg	139.2 ± 6.26*	56.86 ±6.18*	163.88 ± 4.65*	41.93 ±1.10*	127.17 ± 2.61*	11.37 ±1.31*	2.31 ±0.31*
HCD + *Z. simplex* 250 mg/kg	157.76± 9.24*	59.27 ±3.93*	174.69± 5.41*	37.33 ±3.18	145.85± 1.31*	11.85 ±1.54*	3.22 ±0.54*
HCD + *Z. simplex* 500 mg/kg	143.37 ± 8.27*	57.47 ±4.92*	166.81 ± 5.41*	45.74 ± 2.64*	131.31 ± 1.15*	11.49 ±1.32*	2.13 ±0.32*

Data are expressed as mean ± SEM (n= 10). *P 0.05, compared with the HCD group.
TC, Total cholesterol; TG, Triglycerides; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 4: Effect of *Z. simplex* (70% EtOH) extract on liver enzymes, BUN, serum creatinine and total protein in hypercholesterolemic rats for 30 days (10 animals per treatment)

Treatment	Serum ALT(U/L)	Serum AST(U/L)	Serum ALP(U/L)	BUN (mg/dl)	Creatinine (mg/dl)	Total protein (g/dl)
Control	27.39 ±2.79	52.37 ± 3.18	60.72 ± 1.72	32.26 ± 6.3	0.58 ± 0.9	8.26 ± 0.13
HCD	35.34 ±2.83	63.42 ± 1.86	69.53 ± 1.53	33.68 ± 7.66	0.94 ± 1.34 c	7.28 ± 0.88
HCD + SIM 5 mg/kg	28.27 ±1.76*	59.85 ±1.34*	62.76 ±2.30*	30.84 ± 2.66*	0.64 ± 1.66*	10.26 ± 0.61*
HCD + *Z. simplex* 250 mg/kg	30.13 ± 1.83*	61.57 ± 2.87	64.83 ± 4.11	34.17 ± 5.22 *	0.78 ± 1.44*	9.74 ± 0.46
HCD + *Z. simplex* 500 mg/kg	26.55 ±1.44*	58.33 ± 2.18 *	63.38 ± 1.85*	31.61 ± 7.67*	0.67 ± 0.65 *	9.28 ± 0.86 *

Results were expressed as mean ± SEM and analyzed using one-way ANOVA followed by Duncan's post-hoc test. < 0.05 compared to control group, †P < 0.05 compared to HCD group, ‡P *P < 0.05 compared to HCD group

5. CONCLUSION

This study is the first report on the effect of the crude ethanol extract of *Z. simplex* to reduce the serum cholesterol, triglycerides and low-density lipoproteins in hypercholesterolemic male Wistar rats. Being an indigenous plant and considering the variation of associated pharmacological properties; *Z. simplex* has a strong potential in its application in the nutraceutical industry.

Conflict of interest statement

The authors have no declaration of interests in this study.

Formatting of funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

We declare that we have no conflict of interest.

REFERENCES

[1] World Health Organization. Obesity and overweight. Fact Sheet 311. World Health Organization; 2014. [Online] Available from: http://www.who.int/mediacentre/factsheets/fs311/en/index.html. [Accessed on 29 December, 2016].

[2] Li S, Jin S, Song C, Chen C, Zhang Y, Xiang Y, et al. The metabolic change of serum lysophosphatidylcholines involved in the lipid lowering effect of triterpenes from Alismatis rhizoma on high-fat diet induced hyperlipidemia mice. Journal of Ethnopharmacology 2016; **177**: 10–8.

[3] Phondani PC, Bhatt A, Elsarrag E, Horr YA. Ethnobotanical magnitude towards sustainable utilization of wild foliage in Arabian Desert. Journal of Traditional and Complementary Medicine 2016; **6**: 209–18.

[4] Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Natural product reports 2000; **17**:

215–34.

[5] Viktorinova A, Svitekova K, Stecova A, Krizko M. Relationship between selected oxidative stress markers and lipid risk factors for cardiovascular disease in middle-aged adults and its possible clinical relevance. Clinical Biochemistry 2016; **49**: 868–72.

[6] Alsheikh-Ali AA, Kuvin JT, Karas RH. Risk of adverse events with fibrates. Am J Cardiol 2004; **94**: 935–8.

[7] Villaseñor JL. Checklist of the native vascular plants of Mexico. Revista Mexicana de Biodiversidad 2016; **87**: 559–902.

[8] Gujjala S, Putakala M, Ramaswamy R, Desireddy S. Preventive effect of Caralluma fimbriata vs. Metformin against high-fat diet-induced alterations in lipid metabolism in Wistar rats. Biomedicine & Pharmacotherapy 2016; **84**: 215–23.

[9] Agapakis D, Savopoulos C, Kypreos KE, Gbandi E, Iliadis S, Hatzitolios AI, et al. Association of the CETP Taq1B and LIPG Thr111Ile Polymorphisms with Glycated Hemoglobin and Blood Lipids in Newly Diagnosed Hyperlipidemic Patients. Canadian Journal of Diabetes 2016; **40**: 515–20.

[10] Gong J, Fang K, Dong H, Wang D, Hu M, Lu F. Effect of fenugreek on hyperglycaemia and hyperlipidemia in diabetes and prediabetes: A meta-analysis. Journal of Ethnopharmacology 2016; **194**: 260–8.

[11] Boulos L. *Flora of Egypt*. 1st ed. Cairo: Al Hadara Publishing; 2005, p. 236-9.

[12] Migahid AM. *Flora of Saudi Arabia*. 1st ed. Riyadh: University Libraries, King Saud University; 1988, p. 150.

[13] Kirtikar KR, Blatter E, Basu B. *Indian medicinal plants*. 2d ed. Dehra Dun: Bishen Singh Mahendra Pal Singh; 1975, p. 195-200.

[14] Banfield AF. *The Wealth of India*: A Dictionary of Indian Raw Materials and Industrial Products. 1st ed. Science Publishing; 1951, p. 80-9.

[15] Nadkarni KM, Nadkarni AK. *Indian materia medica*. 3rd. ed. Bombay: Popular Prakashan; 1954, p. 120.

[16] Hassanean HA, Desoky EK. *An acylated isorhamnetin glucoside from Zygophyllum simplex*. 1st ed. Pergamon Publishing; 1992, p. 10-15.

[17] Chopra RN, Nayar SL, Chopra IC, Asolkar L V, Kakkar KK, Chakre OJ, et al. *Glossary of Indian medicinal plants*. 1st ed. New Delhi: Council of Scientific & Industrial

Research; 1956, p. 160.

[18] Kakrani HKN, Kakrani PH, Saluja AK. Evaluation of analgesic and anti-inflammatory activity of Ethyl acetate extract of Zygophyllum simplex Linn . herb. International journal of research in phytochemistry and pharmacology 2011; **1**: 180–3.

[19] Daradka HM. Evaluation of Hematological and Biochemical Activity of Ethanolic Extract of Zygophyllum simplex Linn. in Wistar Rats. Pakistan Journal of Biological Sciences 2016; **19**: 179–84.

[20] Gupta P, Bala M, Gupta S, Dua A, Dabur R, Injeti E, et al. Efficacy and risk profile of anti-diabetic therapies: Conventional vs traditional drugs—A mechanistic revisit to understand their mode of action. Pharmacological Research 2016; **113**: 636–74.

[21] Daradka H, Almasad MM, Qazan WS, El-Banna NM, Samara OH. Hypolipidaemic effects of Citrullus colocynthis L. in rabbits. Pakistan Journal of Biological Sciences 2007; **10**: 2768–71.

[22] Shi L, Wang J, Wang Y, Feng Y. MDG-1, an Ophiopogon polysaccharide, alleviates hyperlipidemia in mice based on metabolic profile of bile acids. Carbohydrate Polymers 2016; **150**: 74–81.

[23] Wagner H, Bladt S. *Plant Drug Analysis: A Thin Layer Chromatography Atlas*. 1st ed. Analytica Chimica Acta; 2001, p. 383.

[24] Yu Y-M, Chao T-Y, Chang W-C, Chang MJ, Lee M-F. Thymol reduces oxidative stress, aortic intimal thickening, and inflammation-related gene expression in hyperlipidemic rabbits. Journal of Food and Drug Analysis 2016; **24**: 556–63.

[25] Daradka HM, Abas MM, Mohammad MAM, Jaffar MM. Antidiabetic effect of Artemisia absinthium extracts on alloxan-induced diabetic rats. Comparative Clinical Pathology 2014; **23**. 46-52.

[26] Hrusková J, Danes L, Kliment V. Venezuelan equine encephalomyelitis virus: determination of inhalation LD50 for guinea pigs and mice. Acta virologica 1969; **13**: 203–8.

[27] Chouaïb K, Romdhane A, Delemasure S, Dutartre P, Elie N, Touboul D, et al. Regiospecific synthesis by copper- and ruthenium-

catalyzed azide–alkyne 1,3-dipolar cycloaddition, anticancer and anti-inflammatory activities of oleanolic acid triazole derivatives. Arabian Journal of Chemistry 2016; **15: 589–592.**

[28] Ibrahim AY, Hendawy SF, Elsayed AAA, Omer EA. Evaluation of hypolipidemic Marrubium vulgare effect in Triton WR-1339-induced hyperlipidemia in mice. Asian Pacific Journal of Tropical Medicine 2016; **9**: 453–9.

[29] Riezebos J, Vleeming W, Beems RB, van Amsterdam JG, Meijer GW, de Wildt DJ, et al. Comparison of the antiatherogenic effects of isradipine and ramipril in cholesterol-fed rabbits: II. Effect on regression of atherosclerosis and restoration of endothelial function. Journal of cardiovascular pharmacology 1994; **23**: 424–31.

[30] Bothem KM, Mayes PA. Cholesterol synthesis, transport, and excretion. Harper's Illustrated Biochemistry 27th ed New York, NY: McGraw-Hill 2003; **235**: 285–297.

[31] Niimi M, Yang D, Kitajima S, Ning B, Wang C, Li S, et al. ApoE knockout rabbits: A novel model for the study of human hyperlipidemia. Atherosclerosis 2016; **245**: 187–93.

[32] Lauterbach M, van der Merwe P de W, Keßler L, Pirie MD, Bellstedt DU, Kadereit G. Evolution of leaf anatomy in arid environments – A case study in southern African Tetraena and Roepera (Zygophyllaceae). Molecular Phylogenetics and Evolution 2016; **97**: 129–44.

[33] Katz M. *Study Design and Statistical Analysis: A Practical Guide for Clinicians*. 1st ed. Cambridge: Cambridge University Press; 2006. p. 220-234

[34] Thomas J, El-Sheikh MA, Alfarhan AH, Alatar AA, Sivadasan M, Basahi M, et al. Impact of alien invasive species on habitats and species richness in Saudi Arabia. Journal of Arid Environments 2016; **127**: 53–65.

[35] Bruns CM, Kemnitz JW. Sex hormones, insulin sensitivity, and diabetes mellitus. ILAR journal 2004; **45**: 160–9.

[36] Gundamaraju R, Hwi KK, Singla RK, Vemuri RC, Mulapalli SB. Antihyperlipidemic potential of Albizia amara (Roxb) Boiv. bark against Triton X-100 induced hyperlipidemic condition in rats. Pharmacognosy research 2014; **6**: 267–73.

[37] Qasim M, Abideen Z, Adnan MY, Gulzar S, Gul B, Rasheed M, et al. Antioxidant properties, phenolic composition, bioactive compounds and nutritive value of medicinal halophytes commonly used as herbal teas. South African Journal of Botany 2016; In Press, Corrected Proof.

[38] Zheng Z-G, Zhou Y-P, Zhang X, Thu PM, Xie Z-S, Lu C, et al. Anhydroicaritin improves diet-induced obesity and hyperlipidemia and alleviates insulin resistance by suppressing SREBPs activation. Biochemical Pharmacology 2016; **122**: 42–61.

[39] Ahmad S, Beg ZH. Alleviation of plasma, erythrocyte and liver lipidemic-oxidative stress by thymoquinone and limonene in atherogenic suspension fed rats. Journal of Functional Foods 2013; **5**: 251–9.

[40] Kushi LH, Folsom AR, Prineas RJ, Mink PJ, Wu Y, Bostick RM. Dietary Antioxidant Vitamins and Death from Coronary Heart Disease in Postmenopausal Women. New England Journal of Medicine 1996; **334**: 1156–62.

[41] Matos SL, Paula H de, Pedrosa ML, Santos RC dos, Oliveira EL de, Chianca Júnior DA, et al. Dietary models for inducing hypercholesterolemia in rats. Brazilian Archives of Biology and Technology 2005; **48**: 203–9.

[42] D.S. K, Muthu AK, Manavalan R. Hypolipidemic effect of various extracts of whole plant of Mucuna pruriens (linn) in rat fed with high fat diet. European Journal of Biological Sciences 2010; **2**: 32–8.

[43] Omari-Siaw E, Wang Q, Sun C, Gu Z, Zhu Y, Cao X, et al. Tissue distribution and enhanced in vivo anti-hyperlipidemic-antioxidant effects of perillaldehyde-loaded liposomal nanoformulation against Poloxamer 407-induced hyperlipidemia. International Journal of Pharmaceutics 2016; **513**: 68–77.

[44] Zhen J, Villani TS, Guo Y, Qi Y, Chin K, Pan M-H, et al. Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of Hibiscus sabdariffa leaves. Food Chemistry 2016; **190**: 673–80.

[45] Sodipo A, Abdulrahman FI, S UK, abe, Akinniyi JA. Effects of the aqueous fruit extract of Solanum macrocarpum Linn on the hematological parameters of triton-induced hyperlipidemic rats. African

Journal of Pharmacy and Pharmacology 2011; **5**: 632–9.

[46] Davis RA, McNeal MM, Moses RL. Intrahepatic assembly of very low density lipoprotein. Competition by cholesterol esters for the hydrophobic core. The Journal of biological chemistry 1982; **257**: 2634–40.

[47] Wang Y-M, Zhang B, Xue Y, Li Z-J, Wang J-F, Xue C-H, et al. The mechanism of dietary cholesterol effects on lipids metabolism in rats. Lipids in Health and Disease 2010; **9**: 4.

[48] Mehta LK, Balaraman R, Amin AH, Bafna PA, Gulati OD. Effect of fruits of Moringa oleifera on the lipid profile of normal and hypercholesterolaemic rabbits. Journal of Ethnopharmacology 2003; **86**: 191–5.

[49] Diao S-L, Sun J-W, Ma B-X, Li X-M, Wang D. Influence of crocetin on high-cholesterol diet induced atherosclerosis in rats via anti-oxidant activity together with inhibition of inflammatory response and p38 MAPK signaling pathway. Saudi Journal of Biological Sciences 2016; In Press, Corrected Proof.

[50] Khanna AK, Rizvi F, Chander R. Lipid lowering activity of Phyllanthus niruri in hyperlipemic rats. Journal of Ethnopharmacology 2002; **82**: 19–22.

[51] Del Bas JM, Fernández-Larrea J, Blay M, Ardèvol A, Salvadó MJ, Arola L, et al. Grape seed procyanidins improve atherosclerotic risk index and induce liver CYP7A1 and SHP expression in healthy rats. The FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2005; **19**: 479–81.

[52] Sudhahar V, Kumar SA, Sudharsan PT, Varalakshmi P. Protective effect of lupeol and its ester on cardiac abnormalities in experimental hypercholesterolemia. Vascular Pharmacology 2007; **46**: 412–8.

[53] Fody EP, Bishop ML. Clinical Chemistry: Principles, Techniques and Correlations. 7th ed. London: Lippincott Williams & Wilkins; 2013, p. 784.

[54] Tolins JP, Stone BG, Raij L. Interactions of hypercholesterolemia and hypertension in initiation of glomerular injury. Kidney Int 1992; **41**: 1254–61.

[55] Montilla P, Espejo I, Munoz MC, Bujalance I, Munoz-Castaneda JR, Tunez I. Protective effect of red wine on oxidative stress and

antioxidant enzyme activities in the brain and kidney induced by feeding high cholesterol in rats. Clinical nutrition (Edinburgh, Scotland) 2006; **25**: 146–53.

[56] De Guzman GQ, Ysrael MC, Therese A, Dacanay L, Dimaano JQ. Comparative Hypolipidemic Properties between the Lyophilized Fruit Juice of Morinda citrifolia L. (Rubiaceae) and Lyophilized Commercial Noni Juice in Triton and Atherogenic Diet-Induced Dyslipidemic Rats. International Journal of Pharmacy Teaching & Practices 2013; **4**: 631–5.

[57] El-Newary SA. The hypolipidemic effect of Portulaca oleracea L. stem on hyperlipidemic Wister Albino rats. Annals of Agricultural Sciences 2016; **61**: 111–24.